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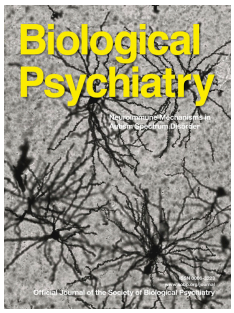

Opposite molecular signatures of depression in men and women

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Opposite molecular signatures of depression in men and women

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Abstract

Background: Major depressive disorder (MDD) affects women approximately twice as often as men. Women are three times as likely to have atypical depression, with hypersomnia and weight gain. This suggests that the molecular mechanisms of MDD may differ by sex.

Methods: To test this hypothesis, we performed a large-scale gene expression meta-analysis across three corticolimbic brain regions, the dorsolateral prefrontal cortex, subgenual anterior cingulate cortex, and basolateral amygdala (N=26 men, 24 women with MDD and sex-matched controls). Results were further analyzed using a threshold-free approach, gene ontology, and cell type-specific analyses. A separate dataset was used for independent validation [N=13 MDD subjects/sex; 22 controls (13 males, 9 females)].

Results: Of the 706 genes differentially expressed in men with MDD and 882 genes differentially expressed in women with MDD, only 21 were changed in the same direction in both sexes. Notably, 52 genes displayed expression changes in opposite directions between men and women with MDD. Similar results were obtained using a threshold-free approach, where the overall transcriptional profile of MDD was opposite in men and women. Gene ontology indicated that men with MDD had decreases in synapse-related genes, whereas women with MDD exhibited transcriptional increases in this pathway. Cell type-specific analysis indicated that men with MDD exhibited increases in oligodendrocyte- and microglia-related genes, while women with MDD had decreases in markers of these cell types.

Conclusions: The brain transcriptional profile of MDD differs greatly by sex, with multiple transcriptional changes in opposite directions between men and women with MDD.

Introduction

Major depressive disorder (MDD) is a leading cause of disability worldwide (1), but its impact differs substantially between sexes. Women are twice as likely to be diagnosed with a single MDD episode, and four times more likely to be diagnosed with recurrent MDD (e.g., (2-7). Women with MDD also report greater illness severity, more symptoms (3, 8-10), and different symptomatology than men. For instance, women are three times more likely to have atypical depression, characterized by hypersomnia and weight gain (11-15). Comorbidity of MDD with other disorders also differs between sexes. For instance, women are more likely to have comorbid anxiety disorders, whereas men are more likely to have comorbid substance use disorders (e.g., (16-19)). Some studies suggest that women have more positive treatment outcomes with selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (20, 21), whereas men seem to respond better to tricyclic antidepressants.

Research suggests dysfunction of the corticolimbic network of mood regulation in MDD. We consider three network nodes, the dorsolateral prefrontal cortex (DLPFC; Brodmann area 9 (BA9)), subgenual anterior cingulate cortex (ACC; BA25), and amygdala (AMY). Structural and functional neuroimaging implicates these regions in MDD [e.g. (22-30)]. Since some studies were performed in only women (24, 31), it is unclear whether results are generalizable to both sexes. Additionally, studies that included both sexes often lacked statistical power to stratify by sex. The idea that these brain regions are differentially affected in men and women with MDD is supported by sex differences in activation during normal emotional states. fMRI studies of non-depressed subjects suggest differential regional during emotion-related tasks, with women having more AMY activation and men more cortical activation [e.g., (32-34)].

Postmortem brain studies report reduced density and number of glial cells in MDD in the DLPFC (35), ACC (36, 37), and AMY (38, 39). Additionally, there is reduced neuron size in DLPFC (36) and ACC (37) in MDD. However, these analyses were not stratified by sex. Gene expression studies on tissue homogenate from postmortem brains have identified sex differences in MDD. In the

ACC, we reported brain derived neurotrophic factor (BDNF)/TrkB expression changes with greater effect in men compared to women with MDD (40). We also reported a more robust reduction in the GABA neuron marker, somatostatin, in the DLPFC, ACC, and AMY of women compared to men with MDD (41). The AMY of women with MDD exhibited a GABA-/BDNF-related dysfunction not seen in men with MDD (41-43). We also found sex differences in cholinergic signaling changes in the AMY of MDD subjects (44). Sex differences in glutamate-related genes were reported in the DLPFC of MDD subjects, with increased expression of glutamate-related genes in women with MDD and decreases in these same genes in men with MDD (45). Finally, Labonte et al recently reported sex-specific transcriptional signatures of depression (46).

Here, we use large-scale gene expression studies, meta-analysis across corticolimbic brain regions, and meta-regression for sex to examine the brain molecular pathology in MDD. Given the sex differences in MDD incidence, symptomatology, and neuroimaging, we hypothesize that the molecular signature of MDD is distinct in men and women.

Materials and Methods

Detailed methods are available in the supplements.

Human subjects and microarray studies

Brain samples were obtained during autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh, USA) after next-of-kin consent using procedures approved by University of Pittsburgh's Institutional Review Board and Committee for Oversight of Research Involving the Dead. Consensus DSM-IV diagnoses were made by an independent committee of experienced clinical research scientists using information from clinical records, toxicology results, and a standardized psychological autopsy. Unaffected comparison subjects were assessed with identical procedures.

50 MDD subjects (26 men, 24 women) and 50 sex-matched unaffected comparison subjects were included. We combined 8 microarray datasets from three brain regions, with half the studies performed in men, half in women (43, 47, 48). Four studies were in ACC (2/sex), two in DLPFC (1/sex), and two in AMY (1/sex). **Tables S1 and S2** contain details on subjects and areas investigated. Group means for age, postmortem interval (PMI), RNA integrity number (RIN), and brain pH were nearly identical and not statistically different.

For replication, we used recently published publically available RNA-seq data (BA11, BA25) generated using brains from a different brain bank (GEO GSE102556; (46)). The effect of MDD was analyzed separately in men and women. We then assessed overlap in DE genes identified in men and women and the percent of overlapping genes that were changed in opposite directions in men and women with MDD.

Meta-analysis of gene expression in MDD

Datasets and meta-analysis methods and results were described previously (49-51). Briefly, we

adopted linear models to account for potential confounding covariates and applied a meta-analysis pipeline to combine studies for identification of MDD-associated genes. A random effects model (REM) was used to detect changes in gene expression by combining effects across studies. We adopted REM separately for the 4 female and 4 male studies. We used $q<0.05$ as the cutoff for differential expression (DE). We then combined all eight studies by REM and used meta-regression to probe for genes that were changed differently in men and women with MDD ($q<0.05$).

Overlap of gene expression profiles in MDD in men and women

Rank-rank hypergeometric overlap test (RRHO): We used RRHO (52) to compare MDD DE genes between men and women. RRHO is a threshold-free algorithm that identifies trends of overlap between two ranked lists of DE genes. The genes are ranked by the –log10 of DE p-value multiplied by the effect size direction. Up, down, and unchanged genes are at the bottom, top, and middle of the list, respectively. A one-sided p-value for the overlap of gene lists from two datasets is calculated according to the hypergeometric distribution.

Spearman's Correlation: We used Spearman's rank-order correlation as a complementary threshold-free method. We compared ranked effect sizes between men and women for all genes.

Gene ontology enrichment analysis

The area under the receiver operating curve (AUROC) statistic was used to measure enrichment of Gene Ontology (GO) groups in a specific gene ranking. This value is equal to the probability that a gene in a GO group will rank higher than a gene not in the group. DE results were ranked from the most significant gene in the negative direction to the most significant gene in the positive direction (signed -log(pvalues)). Mann-Whitney *U* test p-values were calculated. GO groups with 10-200 genes were used (5081 groups).

Cell type-specific analysis

From single-cell transcriptome analysis of healthy human adult cortex, we obtained six lists of the top 21 most enriched genes in transcriptomic-determined cell types (astrocytes, neurons, oligodendrocytes, oligodendrocyte precursors, microglia, and endothelial cells; Table S3 in (53)). The number of genes tested varies because not all 21 genes were assayed in our meta-analysis. We calculated AUROC statistics and Mann-Whitney U p-values for each cell-type list with Bonferroni correction.

Results

Divergent molecular signatures of MDD in men and women

We used large-scale gene expression meta-analysis to probe for sex differences in the brains of men and women with MDD (See strategy in **Figure S1**). We first performed the meta-analysis in each sex separately. There were 706 DE transcripts (252 upregulated, 454 downregulated) in men and 882 DE transcripts (524 upregulated, 358 downregulated) in women. When comparing DE genes in men and women with MDD, 633 of 706 transcripts were found in men only and 809 of 882 transcripts in women only. Interestingly, only 73 genes were DE in both MDD men and women, and 52 of these 73 genes were changed in opposite directions between sexes. Therefore, only 21 DE genes were affected in the same direction in men and women with MDD. Results are summarized in **Figure 1A.** Results are not driven by differences in sex chromosomes, as only 2.5% of genes identified in men and 3.2% of genes identified in women are found on sex chromosomes.

Next, we performed meta-regression on all studies to directly test for expression differences between men and women with MDD. This approach is more stringent than assessing the overlap of DE gene lists identified in men and women separately and is better powered since it includes all eight studies. We identified 1027 genes that were significantly differentially altered in men and women with MDD (**Figure 1A**). A comparison of the meta-regression gene list (1027 genes) indicates that these genes are changed in opposite directions in men and women with MDD (**Figure 2A**). Of these meta-regression genes, 198 and 338 were significant in men or women only, respectively. 52 of the 1027 meta-regression genes were significant for both men and women with MDD, but in opposite directions. These same 52 genes were identified in the previous men/women separate analysis (**Table S3; Figure 2B**). The remaining genes with meta-regression main effect (439) did not reach significance ($q<0.05$) in either sex. Notably, less than 1% of the meta-regression genes were sexually dimorphic in control subjects, indicating that the differential effects in MDD are not driven by baseline sex differences (**Table S4**).

Although most of our male and female MDD studies were performed in separate experiments, one ACC study was performed at the same time in men and women; we directly compared individual gene expression results in this study. We selected three meta-regression genes that were significantly changed in opposite directions in males and females with MDD (*ARPP21*, *P2RY12*, *MTHFR*). We selected an additional 5 genes identified by meta-regression that were significant in only one sex (*CACNA1I*, *ARHGEF3*, *SLCO1A2*, *GABRD*, *CAMK2B*). We confirmed significant interactions of sex and diagnosis for 7/8 genes (**Figure 3A-H**). We also confirmed main effects of diagnosis on expression of *NOL3*, *NUB1*, and *PSMA3*; these genes were changed in the same direction in men and women with MDD (**Figure 3I-K**). To confirm that these changes were consistent across brain regions, we performed an independent qPCR experiment in the AMY for *ARPP21*, *P2RY12,* and *MTHFR*, and found significant interactions of sex and diagnosis for *ARPP21* and *P2RY12* (**Figure S3**). In the AMY, there was a sex difference in *MTHFR*, but no interaction of sex and diagnosis, suggesting that the meta-regression result is driven primarily by the ACC and DLPFC for *MTHFR*.

We confirmed this opposite direction effect in male and female MDD using a separate RNA-seq dataset generated using subjects from a different brain bank (46). We again found very little overlap in DE genes in men and women with MDD (~8%). Notably, ~55% of these overlapping genes changed in opposite directions in men and women with MDD (**Figure 1B-C**; **Tables S5-S6**).

Opposite transcriptional profiles in men and women with MDD

We used a threshold-free approach to validate our divergent gene expression findings in men and women with MDD. Typical DE studies use somewhat arbitrary DE and effect size thresholds to identify relevant genes, which might miss small but reproducible changes. To complement this approach, we used RRHO as an exploratory, threshold-free method to assess patterns of overlap between two DE datasets. For each of the two datasets, RRHO ranks the entire gene list by DE p-value and effect size direction, with one dataset represented on the X-axis and one on the Y-axis. We

first performed RRHO using results from the cross-brain region meta-analysis. We compared the rank ordered gene list generated in depressed men compared to controls (X-axis Figure 4) to the rank ordered gene list generated in depressed women compared to controls (Y-axis Figure 4). **Figure 4A** indicates interpretation of RRHO plots. Consistent with the lack of overlap in DE genes reported above, there was no statistically significant overlap in genes that were upregulated in both men and women with MDD or downregulated in both sexes (**Figure 4B**). However, there was a statistically significant overlap in genes affected in opposite directions in men and women with MDD (**Figure 4B; Figure S4A**). We confirmed this result using Spearman correlation. There was a significant negative correlation in effect sizes for genes in the male-specific dataset to the effect sizes of genes in the female-specific dataset (ρ=-0.130; slope=-0.127; $p=4.39x10^{-41}$), indicating that genes were changed in opposite directions in men and women with MDD.

We also performed RRHO and Spearman correlations separately for each brain region. There was no statistically significant overlap in genes upregulated in both men and women with MDD or downregulated in both sexes for any brain region (**Figure 4C, D, E**). Instead, we observed in the DLPFC and ACC a statistically significant overlap in genes affected in opposite directions in men and women with MDD (**Figure 4C and 3D; Figure S4B and S4C**). We confirmed this negative correlation in effect size direction using Spearman correlation in the DLPFC (ρ=-0.204; slope=-0.197; $p=2.20x10^{-100}$) and ACC (ρ=-0.224; slope=-0.149; $p=4.94x10^{-122}$). In the AMY, there was no statistically significant overlap in genes changed in opposite directions in men and women with MDD (**Figure 4E**). Importantly, RRHO analysis in the replication cohort confirmed these opposite transcriptional profile in male and female depression (**Figure S5**).

Pathway analysis of molecular signatures of MDD in men and women

In men, the DE genes were enriched for synapse-related pathways, inner mitochondrial membrane protein complex, and G-protein coupled amine receptor activity (**Table 1**; top three

pathways are synapse-related, with overlapping genes (**Figure S6**)). Results indicated that genes in these pathways were downregulated in men with MDD.

In women, the DE genes were enriched for pathways related to antigens and mitochondrial function (**Table 2**; top four pathways are antigen-related, with overlapping genes (**Figure S7**)). Genes in these top pathways were downregulated in women with MDD.

Given that some pathways might still be enriched in both men and women with MDD, but to varying degrees, we examined the top pathways identified in each sex in the opposite sex. In other words, a pathway might be enriched in both sexes, but might only be a top pathway in one sex. Interestingly, all five pathways identified in men were also enriched in women (**Table 1**). However, while genes in 4 of the 5 top male pathways were downregulated in men with MDD these same pathways had genes that were upregulated in women with MDD. When we examined the top female identified pathways in men, most were not enriched in men with MDD (**Table 2**).

We next performed GO pathway analysis using genes identified by meta-regression. These genes enriched for regulation of synapse-related pathways, antigen-related pathways, and MHC protein complex (**Table 3**; overlap of genes in the top pathways in **Figure S8**).

Cell type enrichment analysis

We next asked whether sex-specific DE genes were enriched for markers of particular cell types. The goal is to identify candidate cell populations that are likely disrupted in MDD. Results are summarized in **Table 4**. Genes specifically expressed in oligodendrocytes and microglia were upregulated in men with MDD, but downregulated in women with MDD. Genes specifically expressed in astrocytes were upregulated in men with MDD, but unchanged in women with MDD. Neuronal genes were downregulated in men with MDD, but unchanged in women with MDD. We confirmed these cell-type specific results using a single cell dataset generated in mouse cortex (**Table S7**). Together, this cell type enrichment analysis suggests that oligodendrocytes and microglia are oppositely affected in men and women with MDD.

We previously reported reduced expression of oligodendrocyte-specific genes in the AMY of men with MDD (42). Thus, we were surprised that when all three corticolimbic brain regions were combined, there was an increase in expression of oligodendrocyte-specific genes in men with MDD. A closer look at the cell type-specific findings for each brain region in fact confirms our previous AMY finding. While oligodendrocyte-specific genes increase in expression in the DLPFC and ACC of men with MDD, these same genes are decreased in the AMY (**Figure S9A**). Interestingly, the oligodendrocyte-specific genes were upregulated in the AMY, but downregulated in DLPFC and ACC in women with MDD. Together, the cortical patterns for oligodendrocyte-specific genes drives the cross-brain region findings. A closer look at microglia- and neuronal-specific genes showed consistent findings across all three brain regions (**Figure S9B, S9C**).

Discussion

We report almost no overlap in transcriptional changes across corticolimbic brain regions in men and women with MDD, but instead opposite transcriptional changes. Our results suggest that men with MDD have decreases, but women with MDD have increases in synapse-related genes. Immune-related reductions characterized female MDD. Cell type-specific analysis suggests increases in oligodendrocyte- and microglia-specific genes in men with MDD, but decreases in markers of these cell types in women with MDD. Together, these findings point towards distinct, and even opposite molecular changes in MDD in men and women.

Our results are partially consistent with results from a recent publication reporting sex-specific changes in MDD (46). While we also found very little overlap in DE genes in men and women with MDD, our results indicate a high level of transcriptional overlap in genes changed in opposite directions. In fact, we used our statistical methods on the data generated by Labonte et al. (46) and found very similar, but unreported opposite transcriptional results. Brains used in the previous publication were from a different brain bank, supporting the generalizability of our findings. Here, we include results from the AMY, which is not included in Labonte et al. (46). Although consistent with our hypothesis, it is somewhat surprising that these sex-specific molecular changes in MDD were not reported previously. One reason might be because many previous postmortem brain analyses in MDD were performed in mostly (or only) men. Studies that included both sexes mostly did not have sufficient statistical power to stratify by sex, although a few prior reports have hinted at sex differences in MDD (see examples in the Introduction) We believe that our meta-analysis/regression approach gave us the statistical power to investigate larger-scale profiles of molecular changes occurring in the brains of men and women with MDD.

Previous studies reported reduced expression of neuron-specific genes in the AMY of men with MDD and reduced neuronal density in DLPFC (35, 42). Our findings in men with MDD are consistent with those reports. However, we did not find a significant change in neuronal genes in women with MDD. In fact, our results, while not significant after correction for multiple testing, suggest

upregulation of neuron-specific genes in women with MDD. Hence future studies should directly compare neuron and synapse density in the brains of men and women with MDD.

Our findings in men with MDD are consistent with previous reports (that included mostly men) showing reduced markers of synapses, increased markers of inflammation, and reduced spine synapses in the DLPFC (54, 55). Specifically, we report reduced expression of genes related to synapse function and increased expression of microglia-specific genes in men with MDD. Our current findings suggest opposite synapse and inflammation-related changes in women with MDD.

Prior studies demonstrated reduced glial cell densities in DLPFC, ACC, and AMY in MDD (35-37, 39). These studies included both men and women, but did not stratify by sex. Thus, it is unclear whether the findings are sex-specific. Here, we report increases in markers of glia in men, but decreases in women with MDD. Making comparisons between density of glia and changes in expression of glia-specific cells might not be appropriate, as reduced glia density does not necessarily translate into reduced expression of glia-specific genes. Future studies will examine the glial deficits in both sexes, with attention to different glia cell types.

The region-specific findings for oligodendrocyte-specific genes are interesting. In men with MDD, we report increases in oligodendrocyte-specific genes in the DLPFC and ACC, but decreases in expression of these same genes in the AMY. Additionally, in women with MDD, these genes showed decreased expression in the DLPFC and ACC, but increased expression in the AMY (**Figure 4**). Thus, the oligodendrocyte changes in MDD are not only sex-specific, but brain region-specific as well. The cell type-specific findings for microglia-, astrocyte-, and neuronal-specific genes were consistent across brain regions.

The sex differences in MDD that we report might be driven by developmental processes. Developmental exposure to testosterone around the time of birth and through puberty permanently masculinizes the structure of several brain regions (termed organizational effects of hormones). Notably, adolescence is also a sensitive developmental time-period in which there is extensive neuroanatomical, functional, and chemical brain maturation. Events during adolescence that interact

with these developmental processes can increase risk for adult psychopathology. We and others have used various rodent models to manipulate gonadal hormone exposure during critical periods of brain development (perinatal through puberty). For instance, we showed that giving newborn female mice a single dose of testosterone partially masculinizes adult mood-related behavior (i.e., these females had lower anxiety-/depressive-like behaviors in adulthood) (56). Differences in gonadal hormone exposure during development might also influence how the brain responds to a challenge (e.g., chronic stress) in adulthood. Sex differences due to developmental processes might be more relevant to the developmental origins of MDD compared to sex differences emerging in adulthood (e.g., reflecting environmental effects) (57). Since our study includes only adults, we are unable to determine whether the observed sex differences emerge during development or in adulthood.

Our cell type specific and pathway analyses suggest divergent changes in the brains of men and women with MDD. It is quite interesting that in both men and women, the neuronal- and microglial-related changes occur in opposite directions. Women with MDD have decreased markers of immune function and microglia, with increased markers of synaptic function and neurons. On the other hand, men with MDD have increased markers of microglia, with decreased markers of synaptic function and neurons. This opposite direction of effect on microglia and synapses is consistent with a growing literature suggesting that activated microglia have more frequent and prolonged contacts with, and may have increased phagocytosis of, dendritic spines (58). Since our work here is performed in the human postmortem brain, it is unclear whether the synaptic changes observed in MDD (decreased in men, increased in women) are driven by microglia changes, or vice versa. Additionally, it is unclear whether the opposite molecular signatures of MDD in men and women might drive sex differences in MDD symptomatology. To glean more definitive links, follow-up studies in rodent models would perturb immune function in both directions (in both sexes) and assess MDD-associated behavioral domains (e.g., anxiety-, anhedonia-, despair-related behavior).

Limitations of these results are inherent to studies involving human postmortem brains and the heterogeneity of psychiatric cohorts. Although many covariates could affect gene expression

independently of psychiatric diagnosis, our statistical method only included the top two relevant covariates for each gene. This increased our statistical power, but might have ignored additional relevant covariates. We were not sufficiently powered for some cofactors, including recurrent/single episode MDD and comorbid drug abuse. Our meta-analysis/regression approach gave us statistical power to identify consistent molecular changes across brain regions in MDD. However, we note that this method might miss brain region-specific changes important for disease progression. Future studies will use large cohorts of MDD subjects and matched controls, with sufficient statistical power to detect potential sex-specific MDD changes. Although men and women with MDD tend to have differential responses to antidepressants, the medications taken by our subjects were largely similar between men and women, and antidepressant usage was used as a potential cofactor; thus, our results were not driven by different medications between the sexes.

To conclude, our study reveals divergent corticolimbic molecular changes in men and women with MDD. Thus, it follows that potential novel treatments should target sex-specific pathology. For instance, our results suggest that treatments to suppress immune function might be more appropriate for men with MDD, while treatments which boost immune function might be more appropriate for women with MDD. Alternatively, future treatments might aim to target the limited shared pathology present in both men and women with MDD. The implications of MDD cell-specific changes between men and women remain to be further investigated.

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Tables

Table 1. List of top 5 gene ontology pathways identified in men with MDD.[a]

Pathway	Men		Women	
	p-value	AUROC	p-value	AUROC
Regulation of synapse structure or activity[b]	$< 10^{-9}$	0.373 ↓	< 0.01	0.559 ↑
Regulation of synaptic plasticity[b]	$< 10^{-6}$	0.378 ↓	< 0.15	0.544 ↑
Positive regulation of synapse assembly[b]	$< 10^{-5}$	0.312 ↓	< 0.01	0.618 ↑
Inner mitochondrial membrane protein complex	$< 10^{-5}$	0.375 ↓	$< 10^{-6}$	0.347 ↓
G-protein coupled amine receptor activity	$< 10^{-5}$	0.169 ↓	< 0.15	0.622 ↑

Abbreviation: AUC, area under the curve. [a]AUC < 0.5 indicates a pathway is enriched in genes that were downregulated in MDD in that sex. AUC > 0.5 indicates a pathway is enriched in genes that were upregulated in MDD in that sex. [b]The synapse-related pathways have highly overlapping gene lists (see Figure S7). Bold indicates pathways affected in opposite directions in men and women with MDD.

Table 2. List of top 5 gene ontology pathways identified in women with MDD.[a]

Pathway	Women		Men	
	p-value	AUROC	p-value	AUROC
Antigen processing & presentation[b]	$< 10^{-10}$	0.353 ↓	NS	0.522
Antigen processing & presentation of exogenous peptide antigen[b]	$< 10^{-10}$	0.343 ↓	NS	0.515
Antigen processing & presentation of exogenous antigen[b]	$< 10^{-9}$	0.346 ↓	NS	0.511
Antigen processing & presentation of peptide antigen[b]	$< 10^{-9}$	0.354 ↓	NS	0.516
Mitochondrial translational termination	$< 10^{-9}7$	0.337 ↓	<0.05	0.428 ↓

Abbreviation: AUC, area under the curve. [a]AUC < 0.5 indicates a pathway is enriched in genes that were downregulated in MDD in that sex. AUC > 0.5 indicates a pathway is enriched in genes that were upregulated in MDD in that sex. [b]The antigen-related pathways have highly overlapping gene lists (see Figure S8).

Table 3. List of top 5 gene ontology pathways identified by metaR dataset.

Pathway	p-value	Men Effect size	Women Effect size
Regulation of synapse structure or activity[a]	$< 10^{-8}$	- 0.50	0.20
Antigen processing & presentation[b]	$< 10^{-7}$	- 0.003	- 0.50
MHC protein complex[b]	$< 10^{-7}$	0.50	- 1.20
Regulation of synapse organization[a]	$< 10^{-7}$	- 0.46	0.51
Antigen processing & presentation of exogenous peptide antigen[b]	$< 10^{-7}$	- 0.04	- 0.56

[a]The synapse-related pathways have highly overlapping gene lists. [b]The antigen-related pathways pathways have highly overlapping gene lists (see Figure S9).

Table 4. Sex-specific associations of transcriptomic cell-type enriched gene sets.[a]

Cell type	Men		Women	
	q-value	AUC	q-value	AUC
Oligodendrocytes	< 0.005	0.763 ↑	< 0.1	0.319 ↓
Astrocytes	< 0.005	0.734 ↑	NS	0.434
Microglia	< 0.05	0.710 ↑	$< 10^{-4}$	0.134 ↓
Neurons	< 0.05	0.330 ↓	NS	0.525
Oligodendrocyte precursor cells	< 0.12	0.672 ↑	< 0.19	0.682 ↑
Endothelial cells	NS	0.559	NS	0.542

Abbreviation: AUC, area under the curve. [a]AUC > 0.5 indicates a cell type is enriched in genes that were downregulated in MDD in that sex. AUC < 0.5 indicates a cell type is enriched in genes that were upregulated in MDD in that sex. Bold indicates cell-types affected in opposite directions in men and women with MDD.

Figure Legends

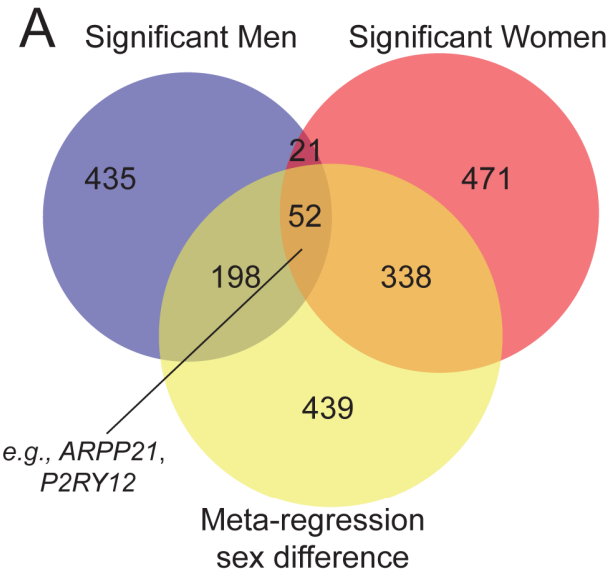
Figure 1. Distinct transcriptional changes in men and women with MDD. (A) Venn diagram displaying overlap in differentially expressed genes in men with MDD, in women with MDD, and in genes identified via meta-regression for sex ($q < 0.05$). We confirmed these results using an independent replication dataset ($p < 0.05$); there was very little overlap in DE genes identified in men and women with MDD in BA25 (**B**) and BA11 (**C**).

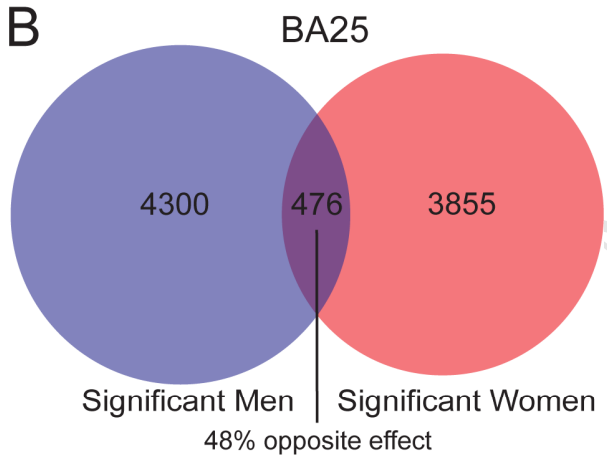
Figure 2. Genes affected in opposite directions in men and women with MDD. (A) Scatterplot indicating the overall pattern of opposite effect size directions for the full meta-regression by sex gene list (1027 genes). **(B)** Heatmap indicating opposite effect sizes of the 52 genes significantly ($q < 0.05$) changed in opposite directions in men and women with MDD. These genes were identified in both the meta-regression dataset as well as in the sex-specific meta-analysis datasets.

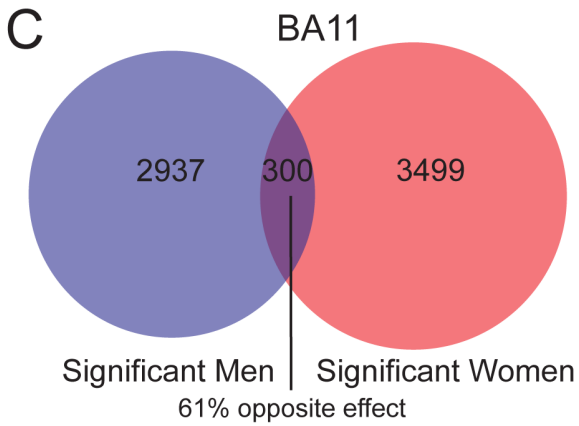
Figure 3. Verification of meta-regression results using arrays in the ACC. The MD2 ACC microarray experiments were performed at the same time in men and women with MDD, allowing us to directly compare expression changes from the microarray studies. There were significant sex x diagnosis interactions for *ARPP21* (**A**), *P2RY12* (**B**), *CACNA1I* (**C**), *SLCO1A2* (**D**), *ARHGEF3* (**E**), *GABRD* (**F**), and *CAMK2B* (**G**). There was a significant main effect of diagnosis on expression of *NOL3* (**I**), *NUB1* (**J**), and *PSMA3* (**K**). (**A**) There was a significant increase in *ARPP21* expression in only women with MDD. (**B**) There was a trend for a decrease in *P2RY12* expression in only women with MDD. (**C**) There was a significant decrease in *CACNA1I* expression in only men with MDD. (**D**) There was a decrease in *SLC01A2* expression in only women with MDD. (**E**) There was an increase in *ARHGEF3* expression in only women with MDD. (**F**) There was a trend for a decrease in *GABRD* expression in only men with MDD. (**G**) There was a decrease in *CAMK2B* expression in only men

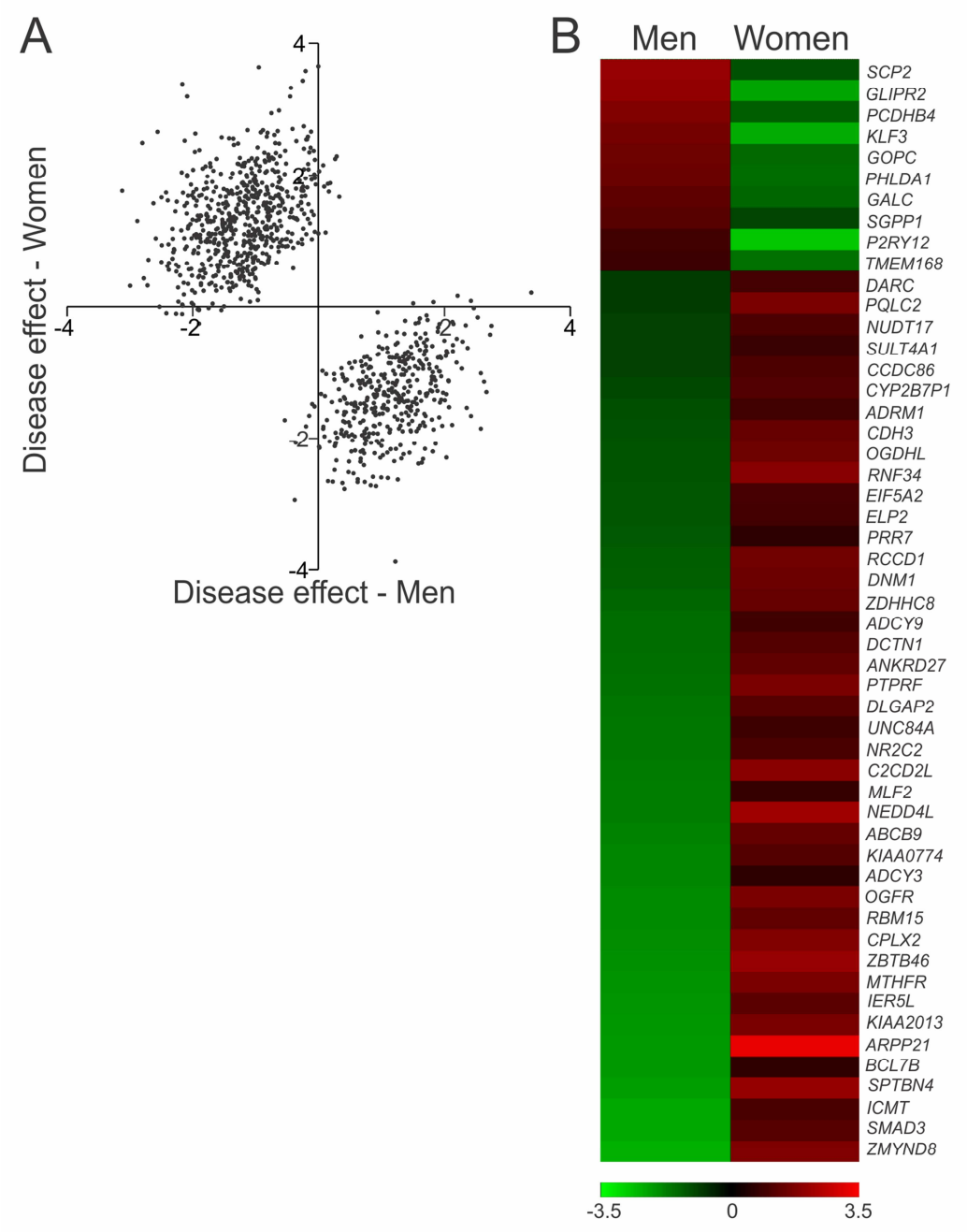
with MDD. (**H**) There was a trend for a decrease in *MTHFR* expression in only men with MDD. (**I**) There was a significant decrease in *NOL3* expression in both men and women with MDD. (**J**) There was a significant increase in *NUB1* expression in both men and women with MDD. (**K**) There was a significant decrease in *PSMA3* expression in men and women with MDD. *, $p < 0.05$; **, $p < 0.01$; #, $p < 0.1$.

Figure 4. Threshold-free differential expression patterns reveal that men and women with MDD have opposite molecular signatures. (A) Schematic indicating interpretation of RRHO plots. A hot spot in the bottom left corner indicates overlap in genes up in both men and women with MDD. A hot spot in the top right corner indicates overlap in genes down in both men and women with MDD. A hot spot in the top left indicates overlap in genes up in men and down in women with MDD. A hot spot in the bottom right indicates overlap in genes down in men and up in women with MDD. Note that in the RRHO plots, the quadrants are not always of equal size; this is due to the fact that there is typically not an even split in the number of genes that are up and down regulated. **(B)** There was no significant overlap in genes that were up in both men and women with MDD or down in both men and women with MDD. However, there was a weak overlap in genes that were changed in opposite directions in men and women with MDD. There was a strong overlap in genes that were affected in opposite directions in the DLPFC (**C**) and ACC (**D**) of men and women with MDD. **(E)** There was no overlap in gene expression profiles in the AMY.


A
Significant Men
Significant Women
435
21
471
52
198
338
439
e.g., ARPP21, P2RY12
Meta-regression sex difference


B
BA25
4300
476
3855
Significant Men
Significant Women
48% opposite effect


C
BA11
2937
300
3499
Significant Men
Significant Women
61% opposite effect


A
Disease effect - Women
Disease effect - Men
4
2
-2
-4
B
Men
Women
SCP2
GLIPR2
PCDHB4
KLF3
GOPC
PHLDA1
GALC
SGPP1
P2RY12
TMEM168
DARC
PQLC2
NUDT17
SULT4A1
CCDC86
CYP2B7P1
ADRM1
CDH3
OGDHL
RNF34
EIF5A2
ELP2
PRR7
RCCD1
DNM1
ZDHHC8
ADCY9
DCTN1
ANKRD27
PTPRF
DLGAP2
UNC84A
NR2C2
C2CD2L
MLF2
NEDD4L
ABCB9
KIAA0774
ADCY3
OGFR
RBM15
CPLX2
ZBTB46
MTHFR
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ARPP21
BCL7B
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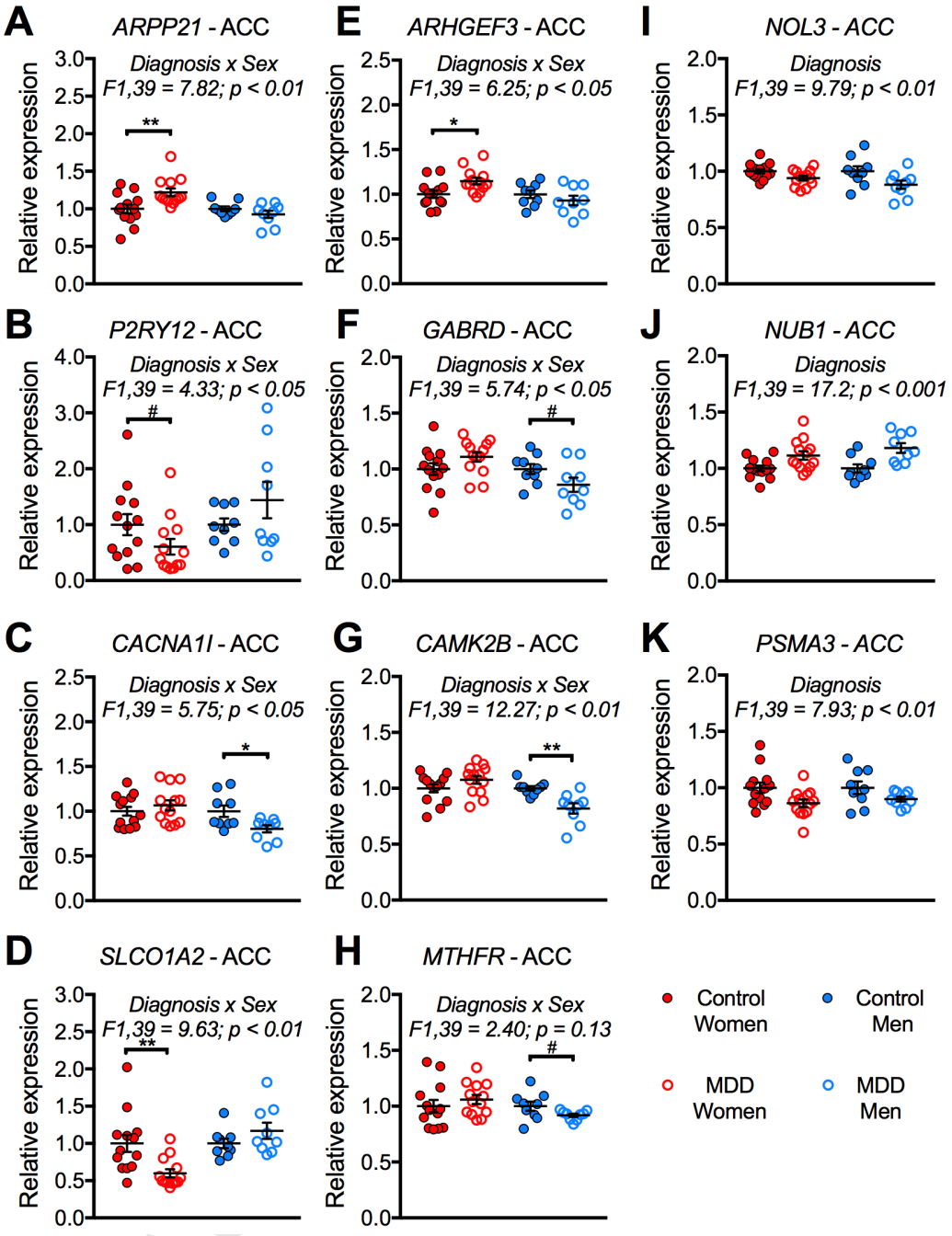
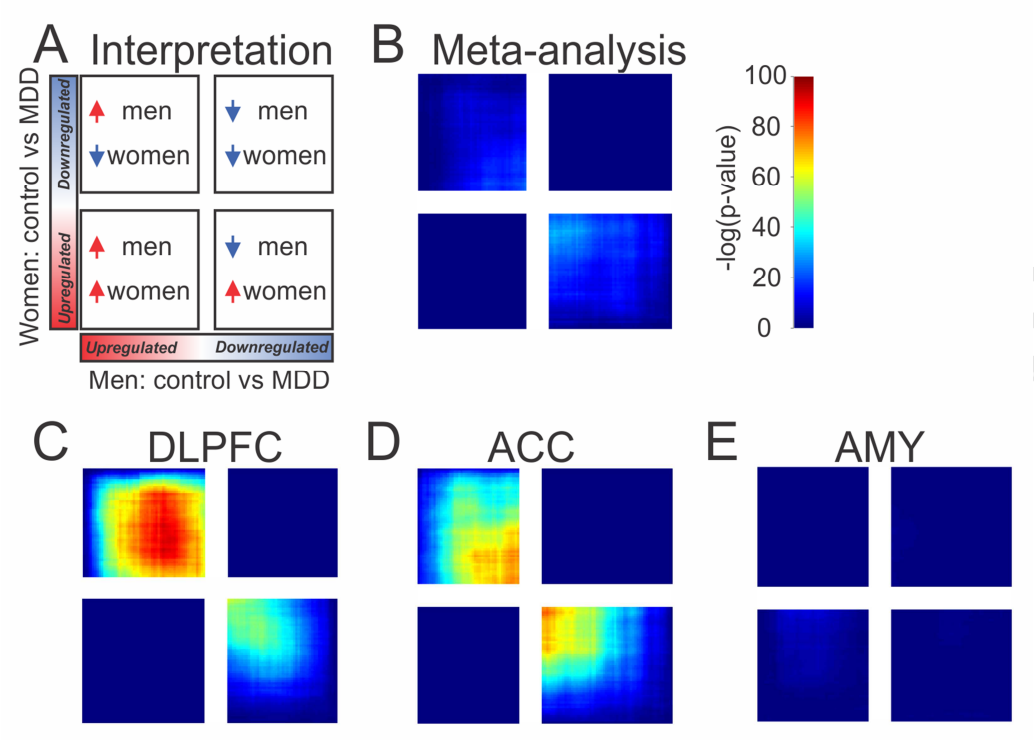
ICMT
SMAD3
ZMYND8
-3.5
0
3.5


A
ARPP21 - ACC
Diagnosis x Sex
F1,39 = 7.82; p < 0.01
**
B
P2RY12 - ACC
Diagnosis x Sex
F1,39 = 4.33; p < 0.05
#
C
CACNA1I - ACC
Diagnosis x Sex
F1,39 = 5.75; p < 0.05
*
D
SLCO1A2 - ACC
Diagnosis x Sex
F1,39 = 9.63; p < 0.01
**
E
ARHGEF3 - ACC
Diagnosis x Sex
F1,39 = 6.25; p < 0.05
*
F
GABRD - ACC
Diagnosis x Sex
F1,39 = 5.74; p < 0.05
#
G
CAMK2B - ACC
Diagnosis x Sex
F1,39 = 12.27; p < 0.01
**
H
MTHFR - ACC
Diagnosis x Sex
F1,39 = 2.40; p = 0.13
#
I
NOL3 - ACC
Diagnosis
F1,39 = 9.79; p < 0.01
J
NUB1 - ACC
Diagnosis
F1,39 = 17.2; p < 0.001
K
PSMA3 - ACC
Diagnosis
F1,39 = 7.93; p < 0.01
Relative expression
Control Women
Control Men
MDD Women
MDD Men



Opposite Molecular Signatures of Depression in Men and Women

Supplemental Information

Supplementary Methods

Gene array data pre-processing

Microarrays were scanned and summarized by manufacturers' defaults. Data from Affymetrix arrays were processed by robust multi-array (RMA) method and data from Illumina arrays by manufacturer's BeadArray software for probe analysis. Batch effects were evaluated and normalized. Oligonucleotide probes (or probesets) were matched to gene symbols using hgu133plus2.db and illuminaHumanv4.db Bioconductor packages.

Individual study analysis

The individual study analysis to detect candidate marker genes involves two major components: random intercept model (RIM) and variable selection. In our previous publication, real data analysis and simulation showed improved statistical power and accuracy when applying the two techniques (1).

Random intercept model (RIM)

To account for the existence of several potential covariates, we applied a random intercept model (RIM). For a given gene *g*, we fit the model:

$$Y_{gik} = \mu_g + \beta_{g0} X_{0ik} + \sum_{l=1}^{L} \beta_{gl} X_{lik} + \alpha_k + \in_{gik}.$$

In the model, Y_{gik} was the gene expression value of gene g (1≤*g*≤*G*) and disease status i(i=1 for control and 2 for MDD) in sample pair k (1≤*k*≤*K*). X_{0ik} was the disease label (1

for MDD, 0 for control). X_{lik} represented values for potential confounding covariate *l* (1≤*l*≤7; 0-1 binary for alcohol dependence, antidepressant drug use and death by suicide, and numerical for age, pH, RIN, and PMI). α_k was the random intercept from a normal distribution with mean zero and variance τ_g^2, which represented the deviation of averaged expression values in the *k*th pair from the average of the whole population. Finally, ϵ_{gik} were independent random noises that followed a normal distribution with mean zero and variance σ_g^2. Under this model, β_{g0} was the disease effect of gene *g* and represented the parameter of major interest. To obtain an MDD-associated differential expression list in each study, we used the likelihood ratio test to assess the p-values of testing $H_o: \beta_{g0} = 0$ (vs $H_A: \beta_{g0} \neq 0$). The p-values were then corrected for multiple comparisons using Benjamini-Hochberg procedure (2). We previously used simulation and real data to demonstrate that including the random effects α_k improved the statistical power (1).

Variable selection for RIM

We have developed and evaluated a variable selection procedure in the random intercept model (namely, RIM_BIC). At most 2 variables were included as covariates for each gene. Specifically, all possible RIM models that included at most two (i.e. 0, 1 or 2) clinical variables were computed and compared. The model with the smallest Bayesian Information Criterion (BIC) (3) value was selected. Here, different sets of covariates were included for each gene based on which covariates were most relevant. In other words, gene A might be confounded by alcohol and RIN, while gene B is confounded by antidepressant and pH. Similar to RIM model, likelihood ratio tests were used to generate p-values of testing $H_o: \beta_{g0} = 0$ in each gene for the selected model by BIC.

Meta-analysis of gene microarray studies

Random effects model (REM) is a popular method for combining effect sizes in meta-analysis.

$$d_{gk} = \mu_g + \alpha_{gk},$$

where d_{gk} is the standardized mean difference (effect size) for gene g $(1 \le g \le G)$ and study k $(1 \le k \le K)$, where G is total number of genes and K is total number of studies. μ_g is true MDD effect for gene g and $\alpha_{gk} \sim N(0, \tau_g^2)$. The goal is to estimate μ_g. (4) described a procedure to combine effect sizes by inverse variance weighting, where the effect size was defined as the standardized mean difference $d = (\overline{Y}_D - \overline{Y}_C)/S_p$, $\overline{Y}_D$ and $\overline{Y}_C$ were the means of MDD and control groups, respectively and S_p^2 indicated an estimation of the pooled variance. The estimated effect size $\widehat{d_{gk}}$ can be estimated by the coefficient of MDD divided by its standard error (i.e., $\hat{\beta}_{gk}/\hat{\sigma}_{gk}$ from RIM model) from single study analysis. Denote the variance of $\widehat{d_{gk}}$ as S_{gk}^2, which can be estimated using delta method. Denote the between-study variance as τ_g^2 which can be estimated by the method of moments suggested by DerSimonian and Larird (5): $\hat{\tau}_g^2 = \max\left\{0, \frac{Q_g - (K-1)}{S_{g1} - \left(S_{g2}/S_{g1}\right)}\right\}$, where $Q_g = \sum_k w_{gk}(\widehat{d_{gk}} - \mu_g)^2$, $\mu_g = (\sum_k w_{gk}\widehat{d_{gk}}) / \sum w_{gk}$, $w_{gk} = \hat{S}_{gk}^{-2}$, $S_{gr} = \sum_k w_{gk}^r$. μ_g and variance of μ_g could be estimated as $\hat{\mu}(\tau_g) = \frac{\sum(\hat{S}_{gk}^2 + \hat{\tau}_g^2)^{-1}\widehat{d_{gk}}}{\sum(\hat{S}_{gk}^2 + \hat{\tau}_g^2)^{-1}}$ and $\mathrm{Var}\left(\hat{\mu}(\tau_g)\right) = \frac{1}{\sum(\hat{S}_{gk}^2 + \hat{\tau}_g^2)^{-1}}$. Under the assumption that the gene expression levels were normally distributed, a z-score to test for differentially-expressed genes was constructed as, $z_g = \frac{\hat{\mu}(\tau_g)}{\sqrt{\mathrm{Var}(\hat{\mu}(\tau_g))}}$, which followed a normal distribution with zero mean and unit variance, under the null. The p-

values of each gene could be calculated and subsequent inferences could be made. We performed Pearson correlation to show the level of statistical agreement across studies. In males, we calculated the Pearson correlation between results from MD2_ACC_M and MD1_ACC_M (both Affy platform), with results represented by scatterplot (**Figure S2A).** In females, we calculated the Pearson correlation between results from MD2_ACC_F and MD3_ACC_F (one Affy platform, one Illumina platform), with results represented by scatterplot (**Figure S2B**).

Meta-regression with variable selection (MetaRG_BIC)

In order to investigate the effect of sex in the random effect model, we adopted a meta-regression model adjusting sex as the only covariate.

$$\mathrm{d_{gk}} = \mu_g + \beta_{gk}\mathrm{X_k} + \alpha_{gk},$$

where μ_g is true MDD effect for gene g and $\alpha_{gk} \sim \mathrm{N}(0, \tau_g^2)$. $\mathrm{X_k}$ is the sex group indicator where $\mathrm{X_k} = 0$ denotes female group and $\mathrm{X_k} = 1$ denotes male group. β_{gk} denotes the sex effect and $\beta_{gk} \neq 0$ indicates the MDD effect in male group and female groups are different. We adopt R package "metaphor" for the estimation procedure.

Sex differences in gene expression in control subjects

We adopted linear models to account for potential confounding covariates (Random Intercept Model). Each gene was fit to linear regression controlling for age, PMI, RIN, and pH. We then used stepwise regression to select the best model, using the covariate with the most significant effect for each gene. Using this model, each gene is tested for the covariate with the most effect on gene expression; that covariate is then used in the model for that gene. This method provides greater statistical power by only accounting for confounding variables that are relevant for each gene. The p-value for significance of the

sex effect is the p-value associated with the t statistic for the coefficient for sex. We then used Benjamini-Hochberg procedure for multiple comparisons within each study to control the false discovery rate (FDR) (2). We then used a q-value cutoff of 0.2 to identify genes that were sexually dimorphic in control subjects. We then asked whether the genes that were sexually dimorphic in controls were present in our meta-regression datasets and calculated the percent overlap.

Confirmation of meta-regression results – replication cohort

We used recently published publicly available RNA-seq data generated using brains from a different brain bank (GEO GSE102556; (6)). Results were confirmed using data from two brain regions (BA11, BA25). We analyzed the effect of MDD separately in men and women. We adopted linear models to account for potential confounding covariates (Random Intercept Model). Each gene was fit to linear regression controlling for RIN, age, medication, and alcohol use (as in the manuscript describing this dataset (6)) In addition, we selected up to two additional covariates using stepwise regression to select the best model. Using this model, each gene is tested for the covariate with the greatest effect on gene expression; that covariate is then used in the model for that gene. This method provides greater statistical power by only accounting for confounding variables that are relevant for each gene. The p-value for significance of the MDD effect is the p-value associated with the t statistic for the coefficient for MDD. We then used a p-value cutoff of 0.05 to identify genes that were DE in MDD subjects (separately in men and women). We then assessed the overlap in DE gene identified in men and women and calculated the percent overlap as well as the percent of overlapping genes that were changed in

opposite directions in men and women with MDD.

Confirmation of meta-regression results – single gene analysis

We confirmed our meta-regression results in two ways. First, since two of the ACC microarray studies (one in men, one in women) were performed at the same time, we could directly compare expression values in men and women. Second, we used qPCR in the AMY of samples obtained from both men and women (controls and MDD). Small qPCR products (80-150 base-pairs) for genes of interest (*ARPP21*, *P2RY12, MTHFR*) were amplified in triplicate on a BioRad CFX96 Touch Real-Time PCR Detection System using standard conditions defined by BioRad (95°C for 2 min followed by 40 cycles: 5s at 95°C, 30s at 60°C). cDNA was amplified in 20µl reactions using SsoAdvanced™ Universal SYBR® Green Supermix according to manufacturer's specifications (450µM primers; BioRad, Hercules, CA, USA). Primer dimers were assessed by amplifying primers without cDNA. Primers were retained if they produced no primer dimers or non-specific signal after 35 cycles and if the product size was as predicted. Results were calculated as the geometric mean of relative intensities compared to two internal control genes (glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and cyclophilin G (*CYCLO*)). These housekeeping genes were previously shown not to be altered in MDD (7). qPCR primers are listed in **Table S8**. Both microarray expression and qPCR datasets were analyzed by 2-way ANCOVA using SPSS (SPSS, Inc., Chicago, IL, USA), with main effects of sex and diagnosis, and interaction of sex and diagnosis. The qPCR data were averaged over three replicates and transformed into arbitrary expression levels ($2^{-\Delta Ct}$), with higher values representing greater expression. To determine relevant covariates to

include in the ANCOVA, Pearson correlation was used to assess the effect of age, postmortem interval, brain pH, RNA ratio, and RIN on gene expression. To determine relevant categorical covariates (alcohol abuse, antidepressant use, death by suicide), gene expression measurements were tested by ANOVA on only MDD subjects. For *ARPP21* array, age was used as a covariate in the ANCOVA. For *ARPP21* qPCR, RNA ratio was used as a covariate in the ANCOVA. For *P2RY12*, *MTHFR*, *SLCO1A2*, *ARHGEF3*, *GABRD*, *CAMK2B*, *CACNA1I*, *NOL1*, *NUB1*, and *PSMA3* array, no covariates were used in the ANCOVA. For *P2RY12* qPCR, RNA ratio, age, and PMI were used as covariates in the ANCOVA. For *MTHFR* qPCR, no covariates were used in the ANCOVA. Statistical significance was set at $p < 0.05$.

Rank-rank hypergeometric overlap (RRHO)

RRHO is a threshold-free algorithm aiming to identify trends of overlap between two biological signatures defined as ranked lists of differential gene expression. We used RRHO to assess overlap in gene lists generated in men with MDD to gene lists generated in women with MDD. RRHO first ranks all genes based on DE p-values and effect size direction. Then, RRHO iterates through different thresholds of the ranked gene list for each dataset and defines “a candidate gene list” to be the amount of genes that are as extreme or more extreme than the current threshold of the same effect size direction. These procedures result in a matrix of hypergeometric p-values whose dimensions are the length of the ranked lists. The hypergeometric p-values are then (1) corrected for multiple comparisons by Benjamini and Yekutieli correction (8), (2) –log10 transformed, and (3) visualized in the heatmap, with each pixel of the heatmap representing an overlap

between two candidate gene lists. Note that in the method described above, we count the candidate gene list to be as extreme or more extreme of the same effect size direction (either top to middle or bottom to middle for a ranked gene list), which is slightly different from the original algorithm (9), where they always count the candidate gene list in the same direction (i.e., overlap in genes changed in the same direction). This approach was particularly relevant for our investigations, as we were interested in overlap in genes that were changed in opposite directions in men and women with MDD. We further split the heatmap into four quadrants using inner boundaries where the effect size direction of the ranked gene list alters. Under this scenario, all four quadrants of the hypergeometric heatmap are biologically meaningful.

Cell-type specific analysis using a mouse dataset

A secondary source of single cell expression data assayed neural cells from mice of both sexes (10). Expression data (number of molecules per cell) was obtained from the Linnarson lab website (https://storage.googleapis.com/linnarsson-lab-www-blobs/blobs/cortex/expression_mRNA_17-Aug-2014.txt). This dataset assayed 3005 cells from the somatosensory (S1) cortex and hippocampus. We used the provided BackSPIN clustering that marked cells as one of 7 major classes ('level1class' in data file) and 47 cell subclasses. We log transformed the provided molecule counts plus one. For each gene, these log scaled values were standardized across all cells. Cells were then grouped by provided 47 subclasses and the average standardized expression value was calculated for each gene. Genes with average standardized expression levels higher than two standard deviations in a given subclass were considered cell-type enriched. The

area under the receiver operating curve (AUROC) statistic was used to measure enrichment of these cell subclass enriched gene lists. The provided cell-type identities or subclasses in the Zeisel dataset was permuted to determine the empirical p-values of the AUROCs (10,000 random assignments of cell subclasses). False discovery rate was used to correct for multiple tests.

Supplementary Figures

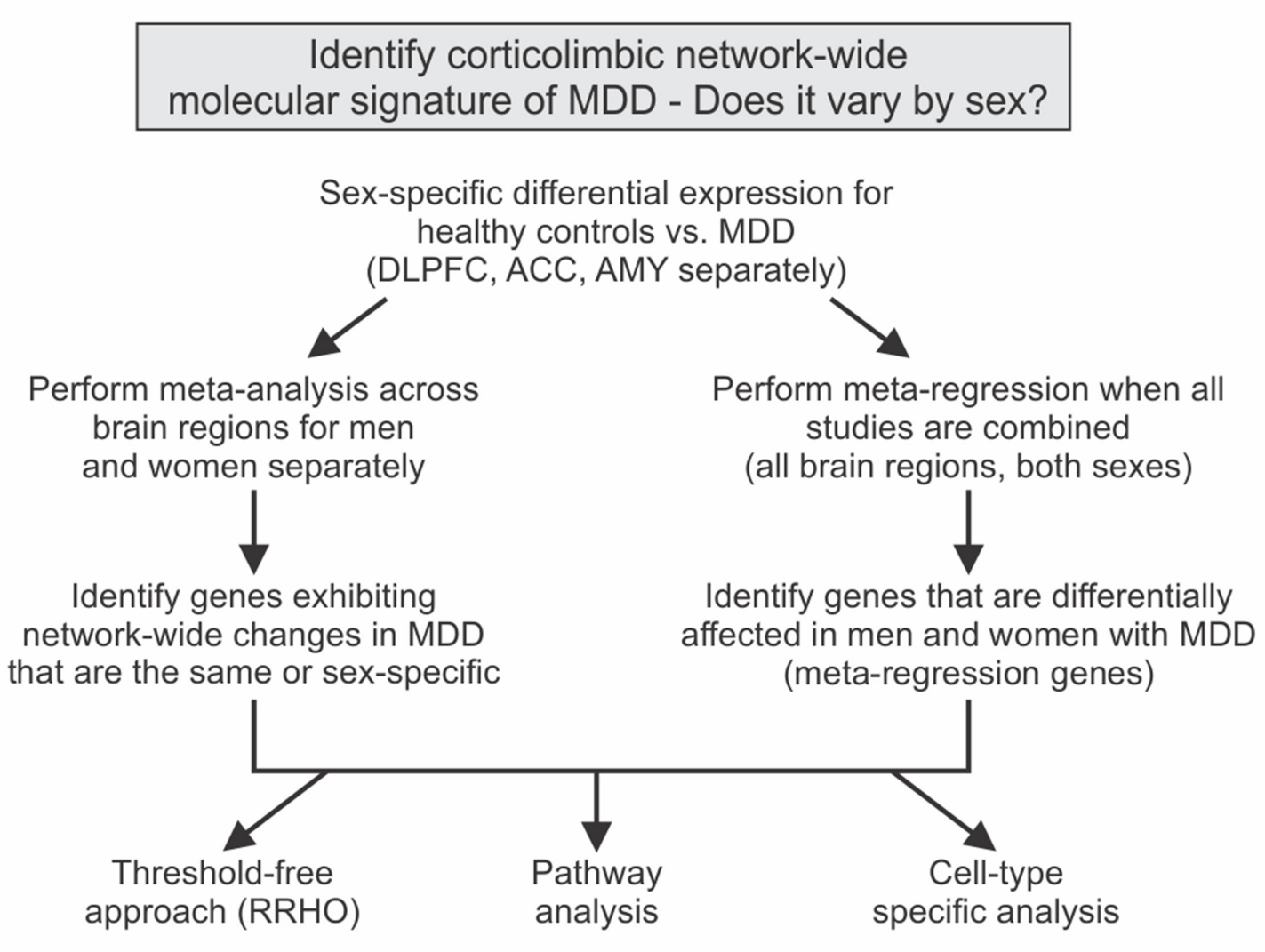


Figure S1. Overview of experimental design for meta-analysis, meta-regression, and downstream analyses.

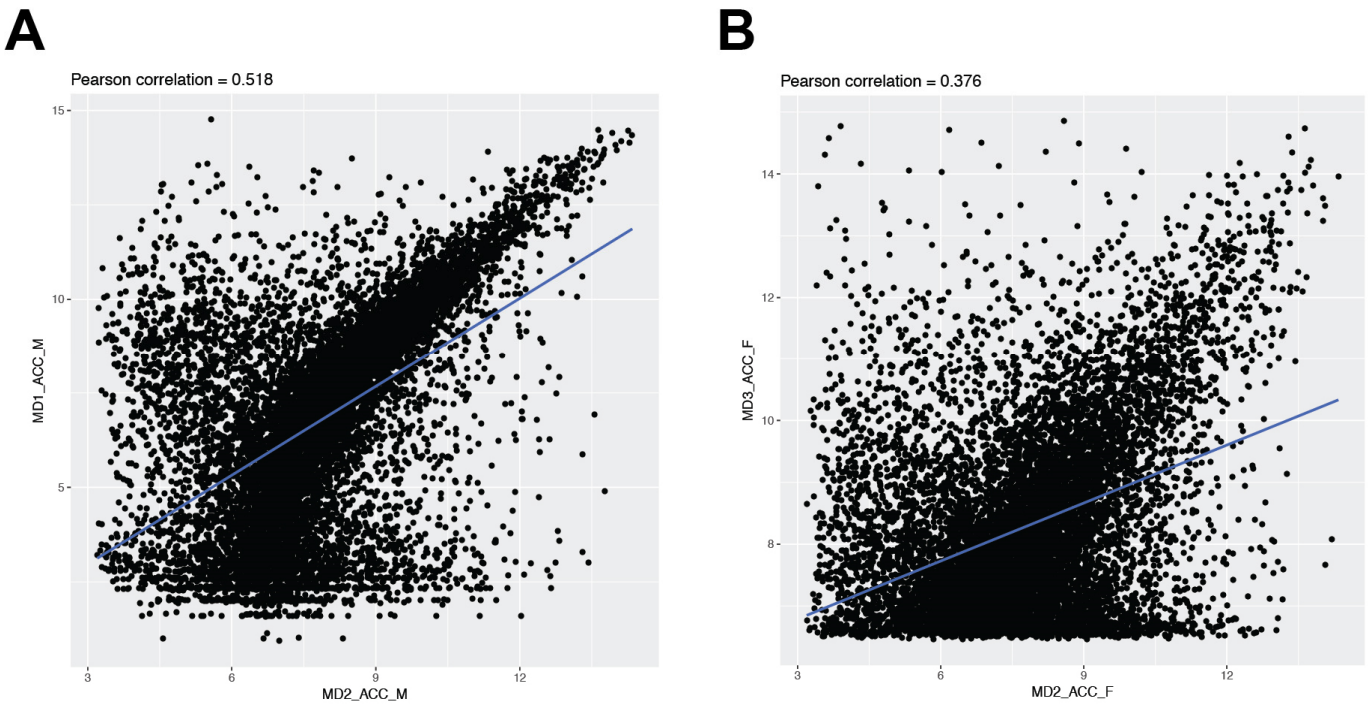


Figure S2. Correlation of gene expression across studies used in meta-analysis. (**A**) In ACC studies performed in males on the same Affymetrix platform, there was a significant correlation of gene expression (Pearson correlation = 0.518). Results are shown by scatterplot. (**B**) In ACC studies performed in females on different platforms (Affymetrix and Illumina), there was a significant correlation of gene expression (Pearson correlation = 0.376). Results are shown by scatterplot.

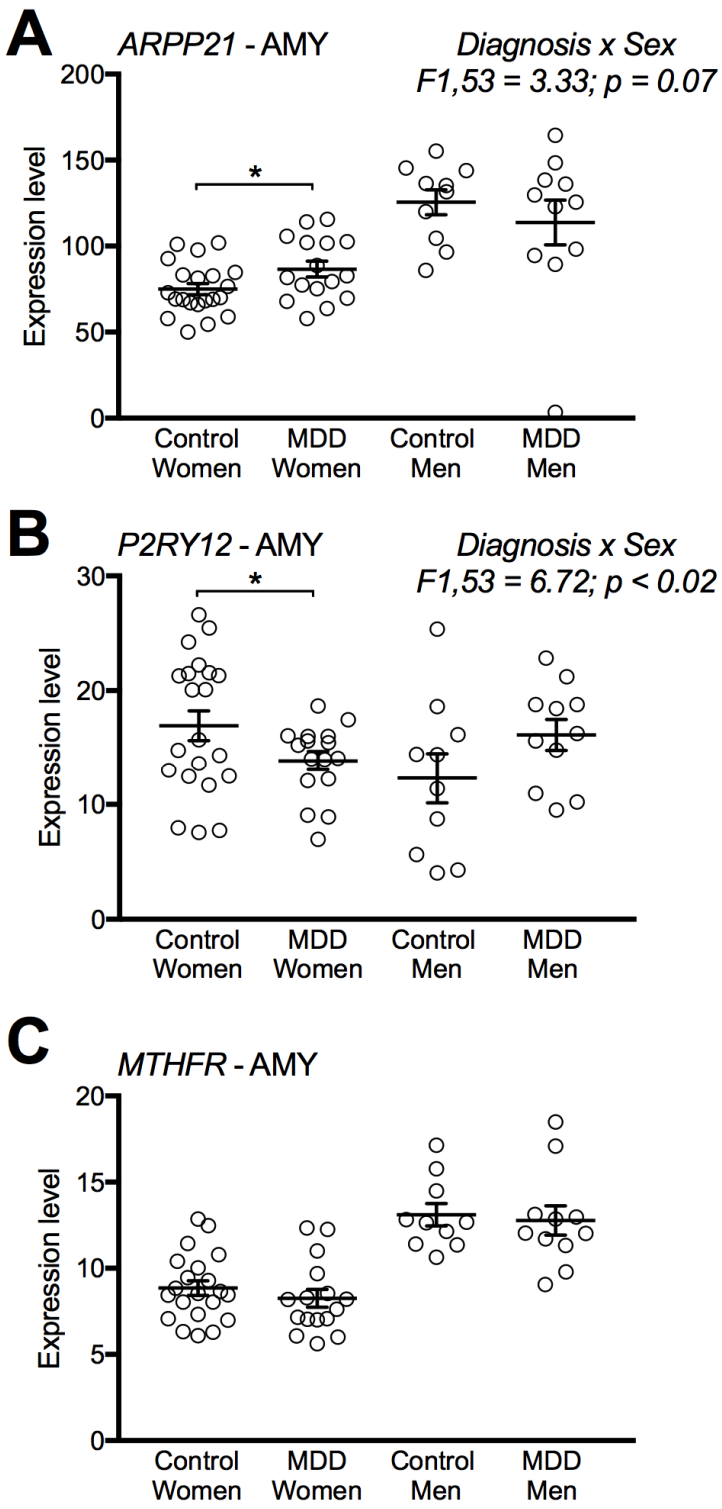


Figure S3. Verification of meta-regression results using qPCR in AMY. There were sex x diagnosis interactions for *ARPP21* (**A**) and *P2RY12* (**B**), but not for MTHFR (C). For *ARPP21* (**A**), there was a significant increase in expression in only women with MDD. For *P2RY12* (**B**), there was a significant decrease in expression in only women with MDD. *, $p < 0.05$.

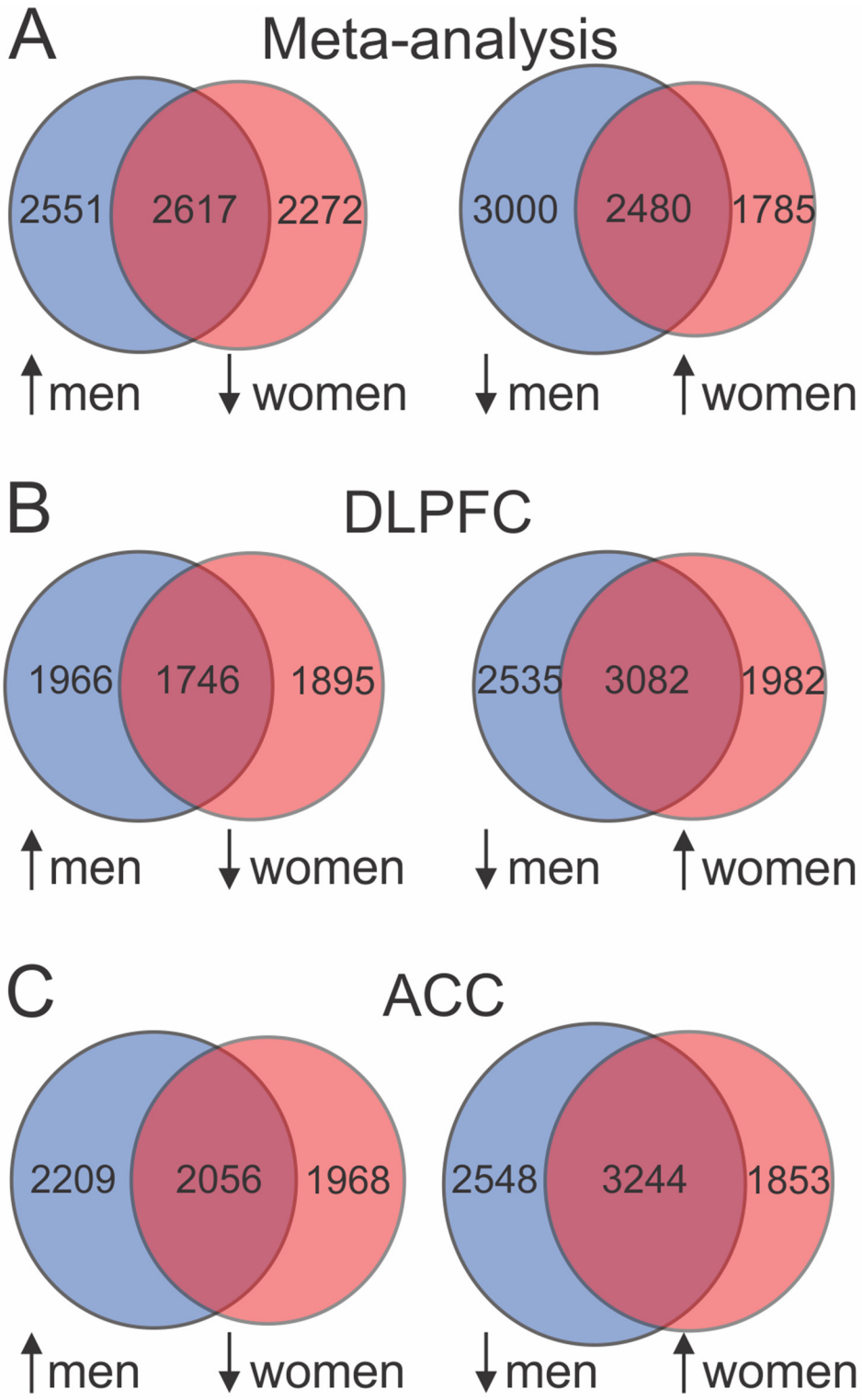


Figure S4. Overlap in opposite molecular profiles in men and women with MDD. (A) Venn diagrams indicating overlap in RRHO-identified genes from the full meta-analysis. **(B)** Venn diagrams indicating overlap in RRHO-identified genes from the DLPFC. **(C)** Venn diagrams indicating overlap in RRHO-identified genes from the ACC.

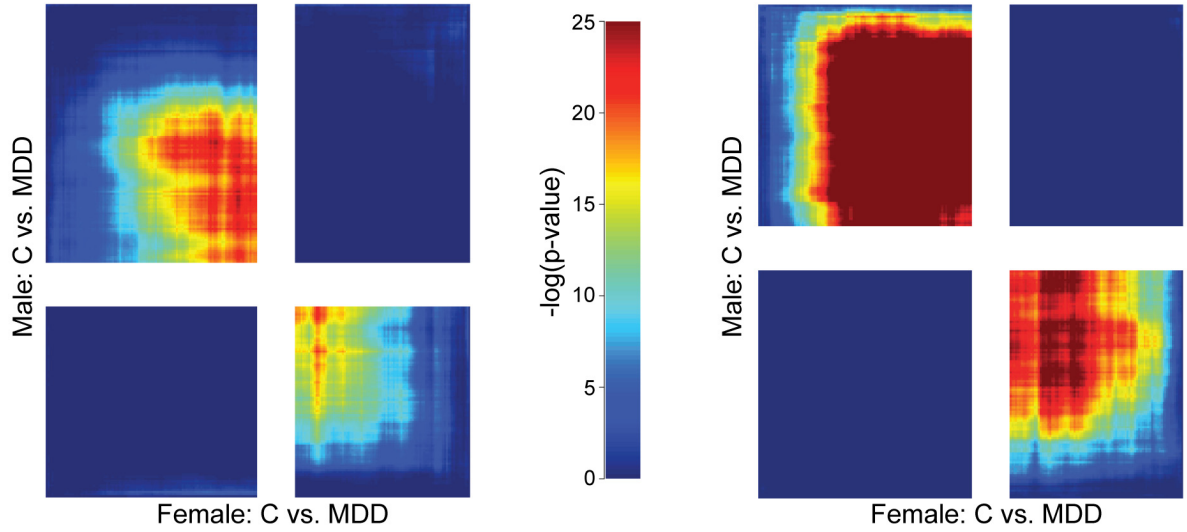


Figure S5. RRHO analysis of replication dataset from Labonte et al. (6) confirmed the opposite transcriptional profile of male and female depression in BA25 (**left**) and BA11 (**right**).

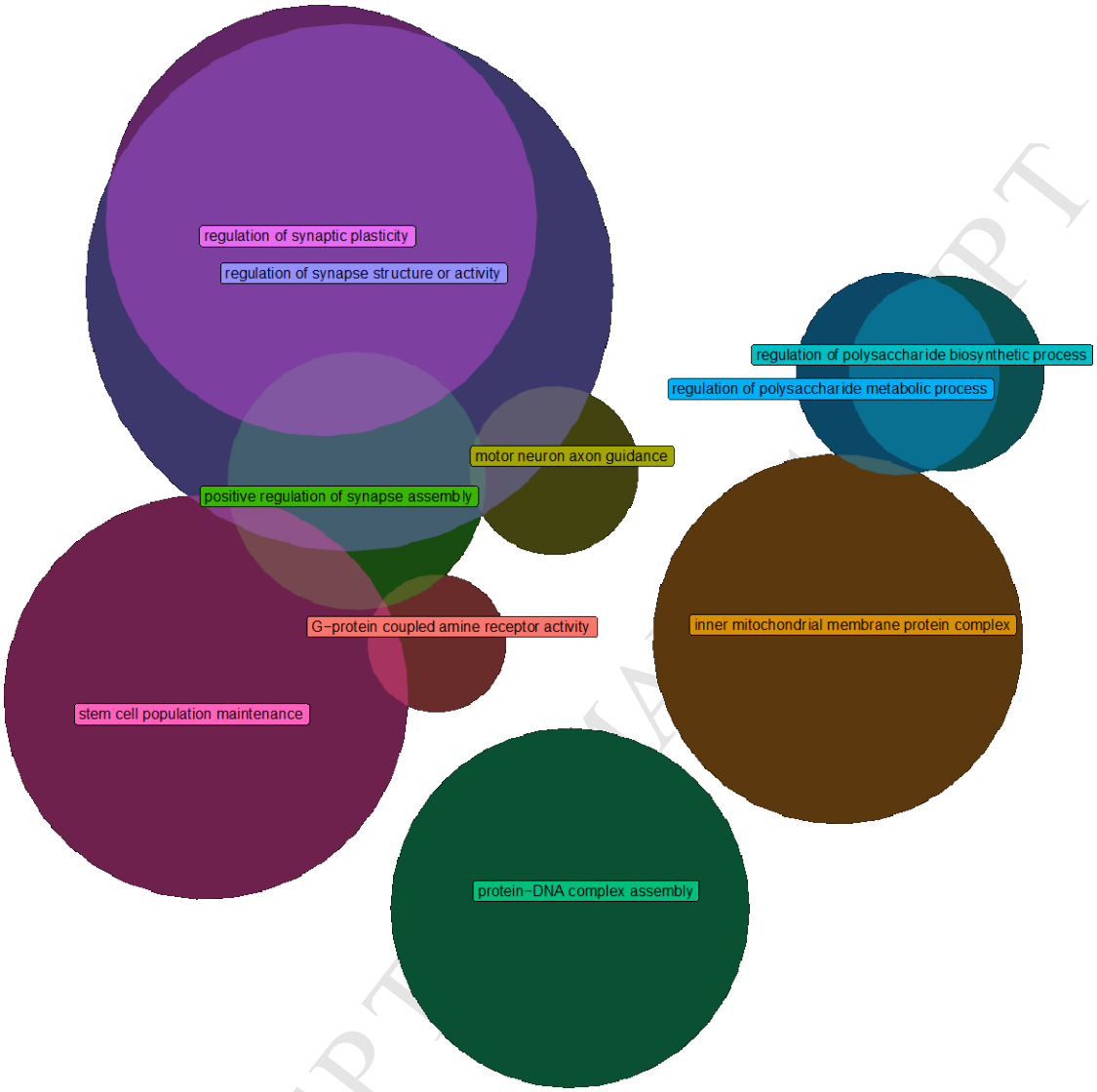


Figure S6. Overlap of top 10 biological pathways identified in men with MDD. Note the high level of overlap in the synapse-related pathways.

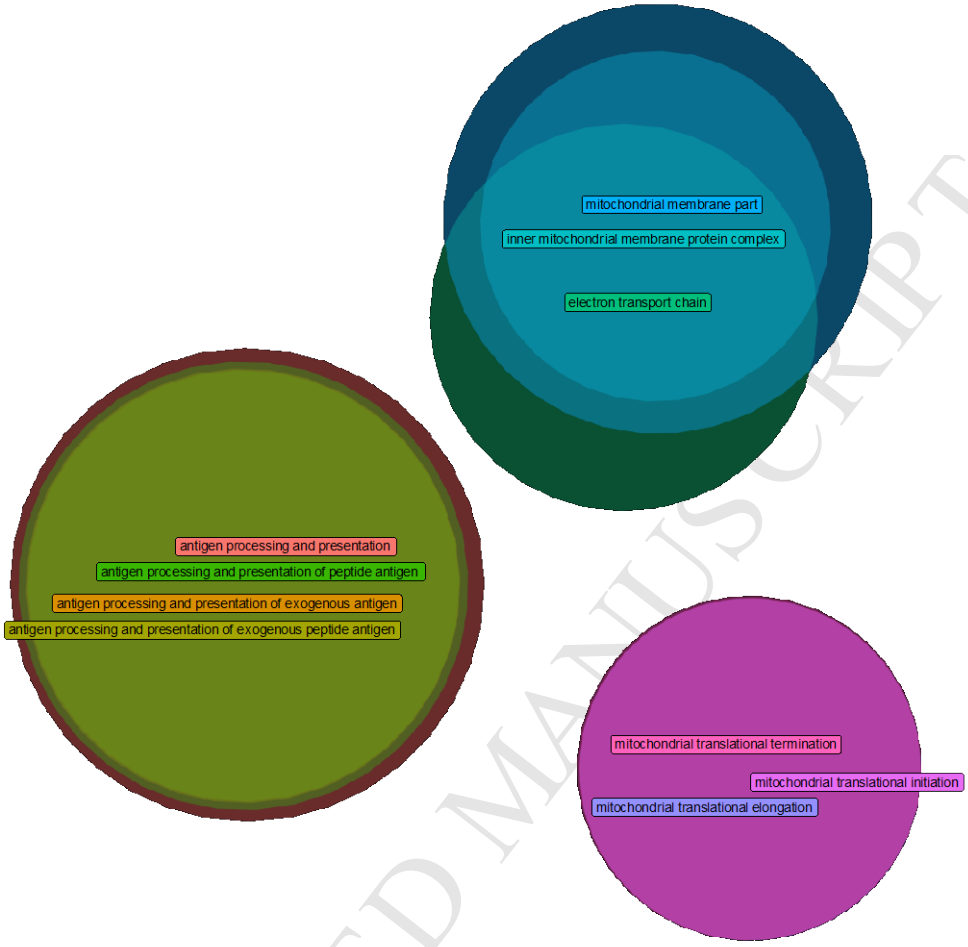


Figure S7. Overlap of top 10 biological pathways identified in women with MDD. Note the high level of overlap in the antigen-related pathways. Additionally, the mitochondrial translation-related pathways overlapped with each other, but not with the mitochondrial membrane-related pathways.

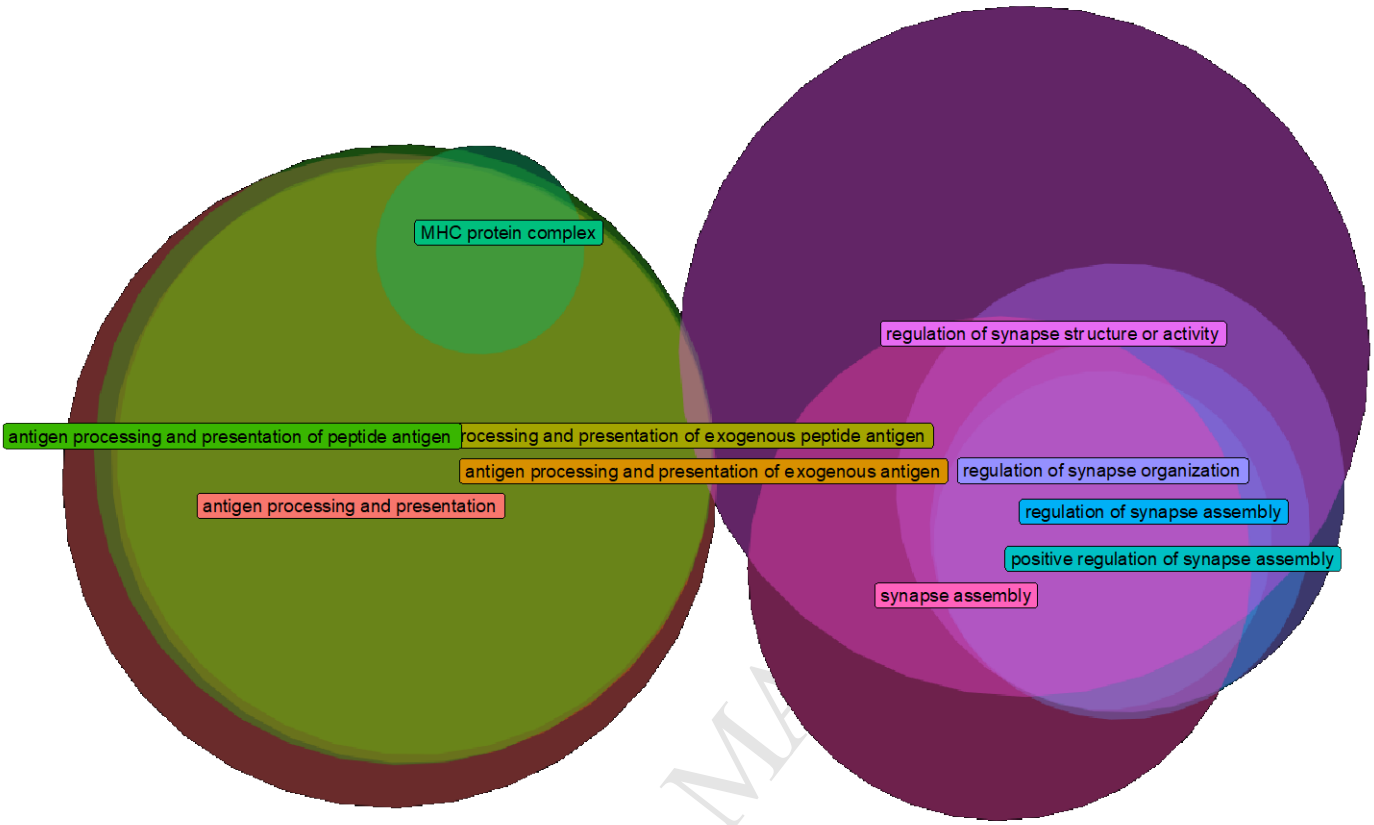


Figure S8. Overlap of top 10 biological pathways identified in the meta-regression dataset. Note the high level of overlap in the antigen-related and MHC pathways. Additionally, the synapse-related pathways are also highly overlapping.

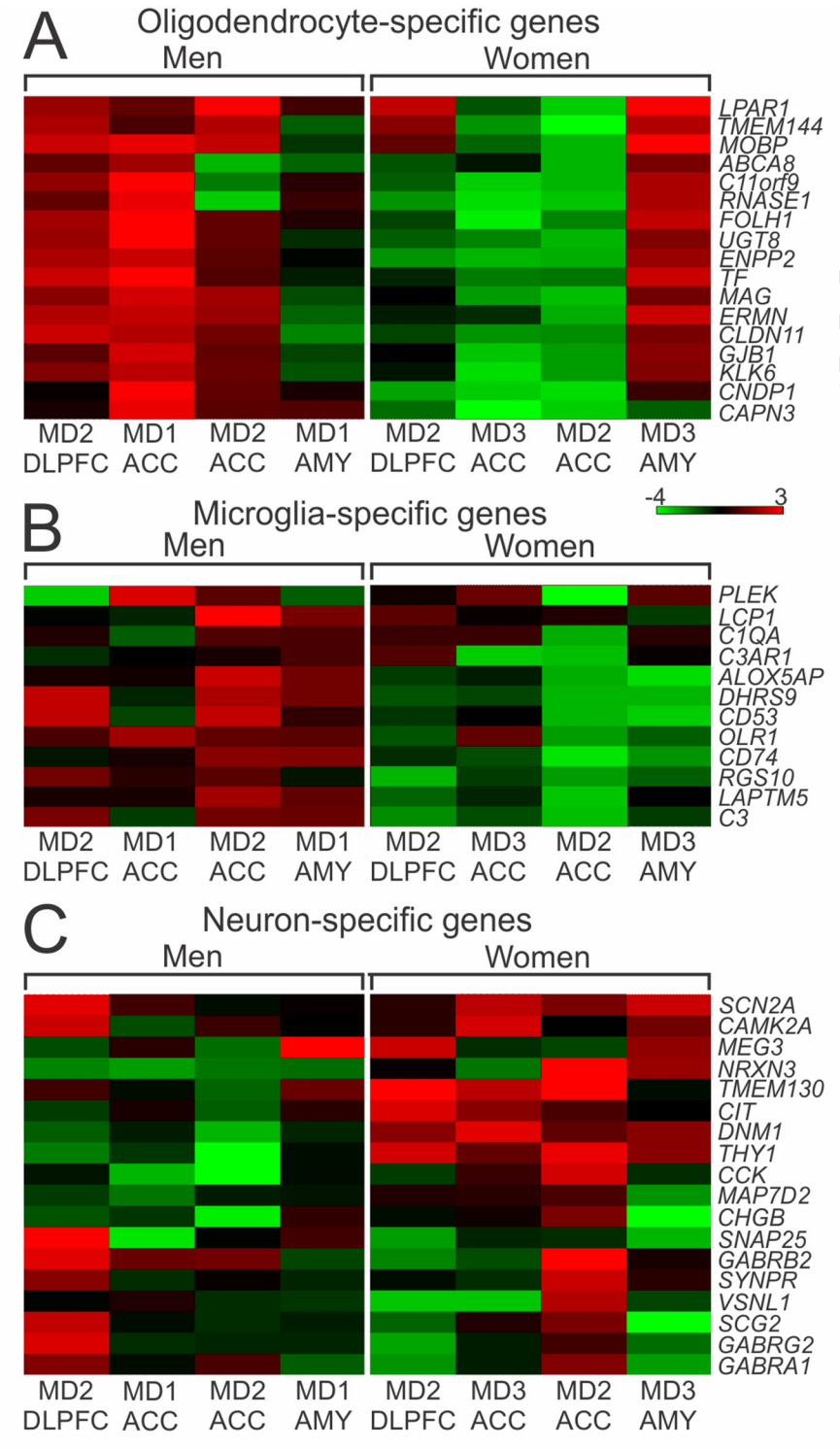


Figure S9. Cell type-specific changes in MDD. (A) There were sex-specific and brain region-specific changes in oligodendrocyte genes. The overall cell type-specific signal when all three brain regions were combined indicated upregulation of oligodendrocyte-specific genes in men with MDD and downregulation of these same genes in women with MDD. This finding was driven by the DLPFC and ACC, with opposite direction of effects

in AMY. **(B)** Across all three brain regions, there were increases in microglia-specific genes in men with MDD, but decreases in these same genes in women with MDD. **(C)** Across brain regions, there were consistent decreases in neuron-specific genes in men with MDD. There were nonsignificant increases in these same neuron-specific genes in women with MDD.

Table S1. Description of eight MDD microarray studies, including data pre-processing and number of genes investigated. See also previous reports on the cohorts and datasets (11-14).

Study name	Sex	Brain region	Sample size	Array platform	# genes before matching	# genes after matching	# genes in common	# genes after filtering
1-MD_ACC_M	Male	ACC	32 (16 pairs)	Affy. HG-U133 Plus 2	40610	19621	16689	10680 genes (20%MV; 20%SD) = 16689 x 0.8 x 0.8
2-MD_ACC_M	Male	ACC	18 (9 pairs)	Affy. HG-U133 Plus 2	53596	19572		
3-MD_ACC_F	Female	ACC	26 (13 pairs)	Affy. HG-U133 Plus 2	53596	19572		
4-MD_ACC_F	Female	ACC	40 (20 pairs)	IlluminaHumanHT-12	48803	25159		
5-MD_AMY_M	Male	AMY	28 (14 pairs)	Affy. HG-U133 Plus 2	40610	19621		
6-MD_AMY_F	Female	AMY	40 (20 pairs)	IlluminaHumanHT-12	48803	25159		
7-MD_DLPFC_M	Male	DLPFC	28 (14 pairs)	Affy. HG-U133 Plus 2	53596	19572		
8-MD_DLPFC_F	Female	DLPFC	30 (15 pairs)	Affy. HG-U133 Plus 2	53596	19572		

Table S2. Demographic and technical details on individual subjects included in each microarray study.

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
615	Control	None	Natural	Ruptured abdominal aortic	M	62	W	7.2	6.4	1.35	7.8	N	N	1				1		1	
789	Control	None	Accidental	Asphyxiation	M	22	W	20.1	7.0	2.00	7.8	N	N	1				1		1	
795	Control	None	Natural	Ruptured abdominal aortic	M	68	W	11.8	6.8	1.60	8.2	N	N	1				1		1	
1031	Control	None	Natural	ASCVD	M	53	W	23.2	6.8	1.50	8.9	N	N	1	1			1		1	
604	Control	None	Natural	Hypoplastic coronary	M	39	W	19.3	7.1	2.11	8.6	N	N	1				1			
685	Control	None	Natural	Hypoplastic coronary	M	56	W	14.5	7.1	1.70	8.1	O	U	1				1			
713	Control	None	Natural	ASCVD	M	58	W	37.5	7.0	1.55	8.4	U	Y	1				1			
736	Control	None	Natural	ASCVD	M	54	W	15.5	6.9	1.56	8.3	N	N	1				1			
852	Control	None	Natural	Cardiac tamponade	M	54	W	8.0	6.9	1.79	9.1	N	Y	1				1			
857	Control	None	Natural	ASCVD	M	48	W	16.6	6.7	2.03	8.9	N	Y	1				1			
1047	Control	None	Natural	ASCVD	M	43	W	13.8	6.6	1.83	9.0	O	N	1				1			
1067	Control	None	Natural	Hypertensive heart	M	49	W	6.0	6.6	1.44	8.2	O	N	1				1			
1086	Control	None	Natural	ASCVD	M	51	W	24.2	6.8	1.36	8.1	N	Y	1				1			
1122	Control	None	Natural	Cardiac tamponade	M	55	W	15.4	6.7	1.40	7.9	O	Y	1				1			
546	Control	None	Natural	ASCVD	F	37	W	23.5	6.7	2.00	8.6	U	U			1			1		1
567	Control	None	Natural	Mitral valve prolapse	F	46	W	15.0	6.8	2.30	8.9	N	U			1			1		1
575	Control	None	Natural	ASCVD	F	55	B	11.3	6.8	1.80	9.6	U	U			1			1		1
1034	Control	None	Natural	Endocardial fibroelastosis	F	23	W	8.5	7.0	2.00	7.8	N	N			1			1		1
1092	Control	None	Natural	Mitral valve prolapse	F	40	B	16.6	6.8	1.70	8.0	O	N			1			1		1
1247	Control	None	Natural	ASCVD	F	58	W	22.7	6.4	1.30	8.4	O	N			1			1		1

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
1282	Control	None	Natural	ASCVD	F	39	W	24.5	6.8	1.30	7.5	N	N			1			1		1
1391	Control	None	Natural	ASCVD	F	51	W	7.80	6.6	1.60	7.1	O	Y			1			1		1
1403	Control	Adjustment disorder with mixed anxiety & depressed mood, in remission (8 months)	Natural	ASCVD	F	45	W	12.3	6.7	1.80	8.2	O	Y			1			1		1
1466	Control	None	Accidental	Trauma	F	64	B	20.0	6.7	2.00	8.8	O	N			1			1		1
1196	Control	None	Accidental	Asphyxiation	F	36	W	14.5	6.4	1.80	8.2	O	N				1		1		1
568	Control	None	Natural	ASCVD	F	60	W	9.5	6.9	1.90	8.7	N	U				1		1		1
627	Control	None	Natural	COPD	F	43	B	14.1	7.1	1.00	7.0	O	N				1		1		
818	Control	None	Accidental	Anaphylactic reaction	F	67	W	24.0	7.1	1.50	8.4	O	N				1		1		
840	Control	Adjustment disorder with depressed mood, current; AAR (20 years remission)	Natural	ASCVD	F	41	W	15.4	6.8	2.00	9.1	N	Y				1		1		
1081	Control	AAR (20 years remission)	Natural	COPD	F	57	W	14.9	6.8	1.80	9.0	B O	N				1		1		
1099	Control	None	Natural	Cardiomyopathy	F	24	W	9.1	6.5	1.90	8.6	O	Y				1		1		
1280	Control	None	Natural	Pulmonary	F	50	W	23.5	6.7	1.30	7.7	U	U				1		1		
1355	Control	None	Natural	Subarachnoid hemorrhage	F	74	W	24.9	6.6	1.90	7.0	O	N				1		1		
10013	Control	None	Accidental	Trauma	F	16	W	9.3	6.7	1.80	9.0	O	N				1		1		
1129	Control	None	Natural	ASCVD	M	54	W	21.0	6.8	1.50	9.0	N	N		1					1	
1317	Control	None	Natural	ASCVD	M	56	W	22.9	6.5	1.20	8.8	O	Y		1					1	
1372	Control	None	Accidental	Asphyxiation	M	37	W	20.5	6.6	1.60	9.0	O	U		1					1	

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
1394	Control	None	Natural	ASCVD	M	45	W	17.3	6.6	1.90	7.3	N	N		1					1	
1439	Control	None	Natural	Subarachnoid hemorrhage	M	56	W	16.1	6.8	2.10	7.7	O	Y		1					1	
1444	Control	None	Natural	Pulmonary	M	46	W	22.0	6.5	2.10	8.4	N	N		1					1	
1462	Control	None	Natural	ASCVD	M	47	W	17.2	6.6	2.00	8.5	N	N		1					1	
612	Control	None	Accidental	Aspiration	M	60	W	9.6	6.8	1.50	9.0	N	U							1	
1214	Control	None	Natural	ASCVD	M	57	W	16.4	6.4	1.70	7.5	O	N							1	
1447	Control	None	Natural	ASCVD	M	51	W	16.2	6.5	1.80	8.5	N	N							1	
686	Control	None	Natural	ASCVD	F	52	W	22.6	7.1	1.90	8.5	O	Y			1					1
731	Control	None	Natural	ASCVD	F	63	W	10.5	6.8	1.60	8.2	N	Y			1					1
1293	Control	None	Accidental	Trauma	F	65	W	18.5	6.6	1.30	7.0	N	N			1					1
1270	Control	None	Accidental	Trauma	F	73	W	19.7	6.7	1.40	7.7	O	N			1					1
634	Control	None	Natural	ASCVD	M	52	W	16.2	7.0	1.90	8.5	N	U	1							
1374	Control	None	Natural	ASCVD	M	43	W	21.7	6.6	1.80	7.2	O	Y		1						
505	MDD	MDD, recurrent, severe without psychotic features; ADC	Suicide	Gunshot	M	57	W	12.8	7.1	1.80	8.9	N	Y	1				1		1	
513	MDD	MDD, recurrent, severe with psychotic features; ODC	Suicide	Hanging	M	24	W	13.1	6.9	1.90	9.0	N	Y	1				1		1	
868	MDD	MDD, recurrent, severe without psychotic features; ADC; OAC	Accidental	Trauma	M	47	W	10.5	6.8	1.50	9.3	N	N	1				1		1	
598	MDD	MDD, single episode, severe without psychotic features; OAR	Suicide	Gunshot	M	69	W	5.9	7.3	1.61	8.8	D O	Y	1				1			

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
600	MDD	MDD, single episode, severe without psychotic features	Suicide	Hanging	M	63	W	9.9	6.7	1.71	7.1	O	N	1				1			
698	MDD	MDD, single episode, severe with psychotic features	Suicide	Hanging	M	59	W	13.0	6.8	1.50	9.0	D O P	N	1				1			
783	MDD	MDD, recurrent, in full remission	Natural	Dissection of the aorta	M	63	W	11.5	6.5	1.36	8.8	O	N	1				1			
809	MDD	MDD, single episode, in full remission	Natural	ASCVD	M	50	W	20.0	6.9	1.52	8.5	D O	Y	1				1			
863	MDD	MDD, single episode, severe without psychotic features	Natural	ASCVD	M	51	W	28.3	7.3	1.52	8.4	N	N	1				1			
926	MDD	MDD, single episode, severe without psychotic features; AAR	Natural	Arteriosclerotic and hypertensive heart	M	56	W	19.0	7.0	1.38	7.3	D O	Y	1				1			
943	MDD	MDD, recurrent, in partial remission; ADC; OAC; ODR	Suicide	Gunshot	M	56	W	15.4	6.6	1.49	8.2	O	Y	1				1			
1001	MDD	MDD, single episode, in full remission	Natural	Arteriosclerotic and hypertensive heart	M	53	W	7.3	6.6	1.38	7.6	O	Y	1				1			
1060	MDD	MDD, single episode, in full remission; AAC	Suicide	Hanging	M	30	W	11.1	6.6	1.32	8.3	O	N	1				1			

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
1049	MDD	MDD, single episode, severe without psychotic features	Natural	Cardiomyopathy	M	48	W	5.4	6.6	1.45	8.4	D O	N					1			
803	MDD	MDD, recurrent, in partial remission	Accidental	Trauma	F	65	W	18.0	7.0	1.90	9.0	D O	N			1			1		1
934	MDD	MDD, recurrent, severe with psychotic features	Natural	ASCVD	F	54	W	17.9	6.5	1.20	8.2	D O	N			1			1		1
967	MDD	MDD, recurrent, moderate; ADC	Natural	ASCVD	F	40	W	22.2	6.6	1.6	7.4	N	Y			1			1		1
986	MDD	MDD, recurrent, severe without psychotic features	Natural	Bronchial asthma	F	53	W	11.9	6.7	1.80	8.8	D O	N			1			1		1
1041	MDD	MDD, recurrent, severe with psychotic features; AAC; ODC	Accidental	Combined drug overdose	F	52	W	10.3	6.5	1.50	8.4	B D O P	Y			1			1		1
1157	MDD	MDD, recurrent, severe without psychotic features	Suicide	Hanging	F	26	W	13.4	6.4	1.50	7.8	D	N			1			1		1
1190	MDD	MDD, recurrent, severe without psychotic features; ADC	Suicide	Asphyxiation	F	47	W	22.3	6.6	1.6	8.0	N	Y			1			1		1
1221	MDD	MDD, recurrent, severe without psychotic features	Natural	Pulmonary thrombosis	F	28	B	24.8	6.6	1.8	7.2	N	N			1			1		1

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
1249	MDD	MDD, recurrent, moderate; ODR	Accidental	Combined drug overdose	F	40	W	11.2	6.5	2.00	9.0	B C D O	Y			1			1		1
1254	MDD	MDD, recurrent, severe without psychotic features	Suicide	Incised wounds	F	39	W	12.8	6.4	1.90	9.0	D	N			1			1		1
1408	MDD	MDD, recurrent, severe without psychotic features; ADC	Accidental	Trauma	F	37	W	15.5	6.6	1.6	7.0	B D O	N				1		1		1
564	MDD	MDD, single episode, severe with psychotic features	Suicide	Hanging	F	56	W	16.8	7.0	1.90	9.2	B D O	Y				1		1		
666	MDD	MDD, single episode, in partial remission	Accidental	Trauma	F	16	W	10.0	7.3	2.00	9.4	D	N				1		1		
1202	MDD	MDD, recurrent, in partial remission	Natural	Pulmonary embolism	F	39	W	11.2	6.4	1.80	8.0	D O	Y				1		1		
1289	MDD	MDD, single episode, mild	Natural	ASCVD	F	46	W	25.0	6.3	1.40	7.3	U	N				1		1		
1315	MDD	MDD, single episode, severe without psychotic features; AAC	Suicide	Hanging	F	28	W	12.4	7.0	1.50	7.9	N	Y				1		1		
1332	MDD	MDD, recurrent, in partial remission; ADR; ODC	Natural	ASCVD	F	46	W	17.5	6.7	1.60	8.9	B D O	Y				1		1		

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
1356	MDD	MDD, recurrent, in partial remission, AAC	Accidental	Intraperitoneal hemorrhage	F	60	W	20.6	6.1	1.80	8.5	D O	N				1		1		
1360	MDD	MDD, single episode, severe without psychotic features; ODC	Suicide	Drowning	F	59	W	18.1	6.4	1.40	7.6	D	Y				1		1		
10028	MDD	MDD, single episode, severe without psychotic features	Suicide	Gunshot	F	72	W	23.1	6.7	1.40	7.0	O	N				1		1		
613	MDD	MDD, recurrent, severe with psychotic features; AAR	Suicide	Gunshot	M	59	W	15.6	7.0	1.90	9.1	O	N	1						1	
1013	MDD	MDD, recurrent, severe without psychotic features	Suicide	Nail gun wound	M	46	W	16.1	6.3	1.50	8.0	N	N		1					1	
1161	MDD	MDD, recurrent, in partial remission, ADR	Natural	ASCVD	M	57	W	15.9	6.6	2.00	7.6	D O	Y		1					1	
1253	MDD	MDD, recurrent, in partial remission; ADC; ODC	Natural	ASCVD	M	58	W	12.5	6.8	1.90	8.1	C D O	Y		1					1	
1261	MDD	MDD, recurrent, moderate; ADC; ODC; OAR	Accidental	Electrocution	M	46	W	22.8	6.6	1.90	8.8	D O	N		1					1	
1312	MDD	MDD, recurrent, severe without psychotic features; ADR; ODC	Accidental	Combined drug overdose	M	51	W	24.6	6.5	1.60	8.5	O	N		1					1	

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
1320	MDD	MDD, recurrent, moderate; ADC	Natural	ASCVD	M	55	W	24.4	6.5	1.30	7.2	N	Y		1					1	
10010	MDD	MDD, recurrent, severe with psychotic features; AAR	Suicide	Amitriptyline overdose	M	42	W	14.3	6.4	1.80	7.6	C D O	N		1					1	
10031	MDD	MDD, recurrent, severe without psychotic features; ADC; OAR	Accidental	Combined drug overdose	M	36	W	20.0	6.8	2.00	8.9	C D P	Y		1					1	
1389	MDD	MDD, recurrent, severe without psychotic features; ADC	Natural	ASCVD	M	61	W	16.0	6.6	1.90	8.4	N	N							1	
10012	MDD	MDD, recurrent, severe without psychotic features; ODC	Suicide	Hanging	M	49	W	24.2	6.4	1.50	8.8	O	Y							1	
1143	MDD	MDD, recurrent, severe without psychotic features; ADR; ODC	Accidental	Combined drug overdose	F	49	W	23.4	6.4	1.80	8.1	B D O	Y			1					1
565	MDD	MDD, recurrent, severe without psychotic features; AAC; ODR	Suicide	Gunshot	F	62	W	12.5	6.9	2.00	9.2	D	N			1					1
1272	MDD	MDD, recurrent, unspecified; ADC; ODC	Accidental	Asphyxiation	F	64	W	12.1	6.6	1.40	7.8	B C D O	Y			1					1

Hu#	Subject Group	DSM-IV Diagnosis	MOD	COD	Sex	Age	Race	PMI	pH	RNA ratio	RIN	Medication ATOD	Tobacco ATOD	1-MD1-ACC-M	2-MD2-ACC-M	3-MD2-ACC-F	4-MD3-ACC-F	5-MD1-AMY-M	6-MD3-AMY-F	7-MD2-DLPFC-M	8-MD2-DLPFC-F
860	MDD	MDD, recurrent; severe with psychotic features	Natural	ASCVD	F	74	W	22.8	7.0	1.20	8.1	B D O P	Y			1					1
619	MDD	MDD, severe without psychotic features; ODR	Suicide	Gunshot	M	55	W	18.8	6.9	1.33	7.9	B D	Y	1							
1226	MDD	MDD, recurrent, severe without psychotic features; ODC; ODR; OAC; OAR	Natural	ASCVD	M	44	W	19.3	6.5	1.70	7.5	N	Y		1						

Abbreviations: AAC, alcohol abuse current; AAR, alcohol abuse remission; ADC, alcohol dependence current; ADR, alcohol dependence remission; ASCVD, arteriosclerotic cardiovascular disease; ATOD, at time of death; B, benzodiazepines; B, black subject; C, anticonvulsants; COD, cause of death; COPD, chronic obstructive pulmonary disease; D, antidepressants; F, female; M, male; MDD, major depressive disorder; MOD, mode of death; N, no medications or no tobacco at time of death; O, other medication(s); OAC, other substance abuse current; OAR, other substance abuse remission; ODC, other substance dependence current; ODR, other substance dependence remission; P, antipsychotics; PMI, postmortem interval in hours; RIN, RNA integrity number; W, white subject; Y, yes.

Table S3. Genes identified via meta-regression which are changed in opposite directions in men and women with MDD.

Gene symbol	*MetaR*	*Men*		*Women*	
	q-value	*Effect Size*	*q-value*	*Effect Size*	*q-value*
C2CD2L	$< 10^{-29}$	- 1.68	$< 10^{-10}$	1.90	$< 10^{-16}$
P2RY12	$< 10^{-24}$	0.92	$< 10^{-4}$	- 2.73	$< 10^{-20}$
PTPRF	$< 10^{-22}$	- 1.56	$< 10^{-7}$	1.68	$< 10^{-15}$
GALC	$< 10^{-22}$	1.28	$< 10^{-6}$	- 1.41	$< 10^{-13}$
PCDHB4	$< 10^{-20}$	1.79	$< 10^{-10}$	- 1.26	$< 10^{-11}$
KLF3	$< 10^{-20}$	1.65	$< 10^{-8}$	- 2.37	$< 10^{-18}$
OGFR	$< 10^{-20}$	- 1.89	$< 10^{-8}$	1.69	$< 10^{-15}$
PHLDA1	$< 10^{-20}$	1.48	$< 10^{-9}$	- 1.50	$< 10^{-12}$
TMEM168	$< 10^{-20}$	0.86	$< 10^{-3}$	- 1.54	$< 10^{-14}$
UNC84A	$< 10^{-18}$	- 1.63	$< 10^{-9}$	0.87	$< 10^{-6}$
ADCY3	$< 10^{-17}$	- 1.83	$< 10^{-11}$	0.64	< 0.05
ZMYND8	$< 10^{-16}$	- 2.42	$< 10^{-13}$	1.74	$< 10^{-6}$
RCCD1	$< 10^{-15}$	- 1.27	$< 10^{-6}$	1.56	$< 10^{-13}$
CDH3	$< 10^{-13}$	- 1.13	$< 10^{-4}$	1.41	$< 10^{-12}$
ARPP21	$< 10^{-13}$	- 2.08	$< 10^{-8}$	3.19	$< 10^{-6}$
GLIPR1	$< 10^{-13}$	2.04	$< 10^{-11}$	- 2.29	$< 10^{-6}$
SMAD3	$< 10^{-12}$	- 2.32	$< 10^{-6}$	1.19	$< 10^{-9}$
CCDC86	$< 10^{-12}$	- 0.89	$< 10^{-4}$	1.08	$< 10^{-8}$
IER5L	$< 10^{-12}$	- 2.03	$< 10^{-7}$	1.26	$< 10^{-9}$
MTHFR	$< 10^{-12}$	- 2.01	$< 10^{-4}$	1.70	$< 10^{-13}$
SPTBN4	$< 10^{-12}$	- 2.17	$< 10^{-8}$	2.05	$< 10^{-7}$
ADCY9	$< 10^{-11}$	- 1.49	$< 10^{-9}$	0.89	$< 10^{-4}$
KIAA0774	$< 10^{-10}$	- 1.83	$< 10^{-6}$	1.16	$< 10^{-7}$
ICMT	$< 10^{-10}$	- 2.31	$< 10^{-4}$	1.04	$< 10^{-7}$
NEDD4L	$< 10^{-10}$	- 1.72	$< 10^{-10}$	2.19	$< 10^{-3}$
OGDHL	$< 10^{-10}$	- 1.15	< 0.05	1.54	$< 10^{-14}$
KIAA2013	$< 10^{-10}$	- 2.07	$< 10^{-5}$	1.69	$< 10^{-7}$
RNF34	$< 10^{-10}$	- 1.17	$< 10^{-6}$	1.86	$< 10^{-3}$
CPLX2	$< 10^{-10}$	- 1.94	$< 10^{-11}$	1.79	< 0.05
SCP2	$< 10^{-9}$	2.08	< 0.05	- 1.13	$< 10^{-8}$
ZDHHC8	$< 10^{-9}$	- 1.42	$< 10^{-7}$	1.42	$< 10^{-6}$
RBM15	$< 10^{-9}$	- 1.89	$< 10^{-9}$	1.33	$< 10^{-3}$
BCL7B	$< 10^{-9}$	- 2.09	< 0.05	0.66	$< 10^{-3}$
ABCB9	$< 10^{-9}$	- 1.78	$< 10^{-6}$	1.40	$< 10^{-5}$
MLF2	$< 10^{-9}$	- 1.71	$< 10^{-9}$	0.75	< 0.05
ZBTB46	$< 10^{-9}$	- 1.99	$< 10^{-5}$	2.09	$< 10^{-3}$
GOPC	$< 10^{-9}$	1.53	$< 10^{-8}$	- 1.44	$< 10^{-4}$
ANKRD27	$< 10^{-8}$	- 1.52	$< 10^{-8}$	- 1.33	$< 10^{-3}$
EIF5A2	$< 10^{-8}$	- 1.18	$< 10^{-6}$	1.01	$< 10^{-3}$
DNM1	$< 10^{-8}$	- 1.30	$< 10^{-3}$	1.49	$< 10^{-7}$
DARC	$< 10^{-7}$	- 0.82	< 0.05	0.97	$< 10^{-6}$
NR2C2	$< 10^{-7}$	- 1.65	$< 10^{-5}$	1.01	$< 10^{-4}$
ADRM1	$< 10^{-7}$	- 1.07	$< 10^{-5}$	0.90	$< 10^{-3}$
DCTN1	$< 10^{-7}$	- 1.50	$< 10^{-3}$	1.16	$< 10^{-6}$
PQLC2	$< 10^{-7}$	- 0.84	$< 10^{-3}$	1.71	< 0.05
CYP2B7P1	$< 10^{-6}$	- 0.99	$< 10^{-5}$	1.16	$< 10^{-3}$
PRR7	$< 10^{-6}$	- 1.23	$< 10^{-3}$	0.64	< 0.05
DLGAP2	$< 10^{-6}$	- 1.60	< 0.05	1.19	$< 10^{-3}$
ELP2	$< 10^{-5}$	- 1.19	< 0.05	0.94	$< 10^{-3}$
NUDT17	$< 10^{-5}$	- 0.88	< 0.05	1.07	$< 10^{-3}$
SGPP1	$< 10^{-5}$	1.19	< 0.05	- 0.97	$< 10^{-3}$
SULT4A1	$< 10^{-5}$	- 0.88	$< 10^{-3}$	0.82	$< 10^{-3}$

Table S4. Overlap in DE genes from male MDD and female MDD with genes that are DE between male and female healthy controls.[a]

	DE genes for meta-regression that are also DE at baseline	DE genes in males with MDD that are also DE at baseline	DE genes in females with MDD that are also DE at baseline	DE genes in opposite directions in male and female MDD that are also DE at baseline
ACC	10/1027	6/706	10/882	1/52
DLPFC	9/1027	3/706	9/882	1/52

[a]For baseline sex difference analysis, a cutoff of q < 0.2 was used to identify genes that were sexually dimorphic in control subjects.

Table S5. Sex-specific depression changes confirmed using a different brain bank cohort.[a]

	DE genes in women with MDD	DE genes in men with MDD	Overlap of DE genes in men and women with MDD	% genes changed in opposite directions in men and women with MDD
BA11	3798	3237	299	61%
BA25	4331	4776	476	48%

[a]We used recently published publically available RNA-seq data generated using brains from a different brain bank (GEO GSE102556; (6)). p < 0.05 was used as a DE cutoff.

Table S6. Replication cohort: top 10 transcripts significantly changed in opposite directions in men and women with MDD.

BA11					BA25				
Gene symbol	*Men*		*Women*		*Gene symbol*	*Men*		*Women*	
	Effect size	*p-value*	*Effect size*	*p-value*		*Effect size*	*p-value*	*Effect size*	*p-value*
FCGR1C	-1.840	$>10^{-4}$	1.81	$>10^{-4}$	*EDAR*	-1.45	>0.05	1.48	>0.05
RP11-462G2.2	-1.18	$>10^{-3}$	2.15	$>10^{-4}$	*RP11-536O18.2*	-1.43	$>10^{-3}$	1.02	>0.05
MYBPH	-1.14	>0.05	1.78	$>10^{-4}$	*HLA-DOB*	-1.39	>0.05	2.12	$>10^{-3}$
SNORD53_SNORD92	1.27	$>10^{-5}$	-1.64	>0.05	*RP11-370B11.1*	-1.37	$>10^{-3}$	1.12	>0.05
AC097721.1	-1.50	$>10^{-3}$	1.21	>0.05	*KCNE1L*	-1.35	>0.05	1.68	>0.05
RPS3AP25	-0.82	>0.05	1.82	$>10^{-3}$	*AC104088.1*	-1.34	$>10^{-3}$	1.21	$>10^{-3}$
CD69	-0.86	>0.05	1.67	>0.05	*RP4-660H19.1*	-1.32	>0.05	0.80	>0.05
KRT8P13	-1.00	>0.05	1.53	>0.05	*IRX6*	-1.31	>0.05	1.38	>0.05
RP11-307C19.3	0.79	>0.05	-1.73	$>10^{-3}$	*CTD-2623N2.11*	-1.24	>0.05	1.36	$>10^{-3}$
RP11-159C21.4	-1.17	$>10^{-3}$	1.34	>0.05	*AC110754.3*	-1.22	$>10^{-3}$	1.13	>0.05

Table S7. Sex-specific associations of transcriptomic cell-type enriched gene sets using mouse reference dataset.[a]

Cell type	Men		Women	
	p-value	AUC	p-value	AUC
Astro2	< 0.005	0.683 ↑	NS	0495
Astro1	< 0.005	0.681 ↑	NS	0.497
Mgl2	< 0.05	0.594 ↑	$< 10^{-4}$	0.377 ↓
S1PyrL4	< 0.2	0.602 ↑	< 0.005	0.602 ↑
Epend	< 0.01	0.612 ↑	NS	0.518
Oligo5	< 0.01	0.718 ↑	NS	0.408

Abbreviation: AUC, area under the curve. [a]AUC > 0.5 indicates a cell type is enriched in genes that were downregulated in MDD in that sex. AUC < 0.5 indicates a cell type is enriched in genes that were upregulated in MDD in that sex. Bold indicates cell-types affected in opposite directions in men and women with MDD.

Table S8. Primers used in qPCR studies.

Gene	Forward	Reverse
ARPP21	5' TAC CAC CGG CAC TTA CAA 3'	5' GGG AAG CGA TAC AAT CCA 3'
P2RY12	5' GTG TCA AGT TAC CTC CGT CAT A 3'	5' TAA ATG GCC TGG TGG TCT 3'
MTHFR	5' TTG TGT TTG GTT TGG TGG T 3'	5' CAT CGG TCA GTC CCT CTC 3'
GAPDH	5' TGC ACC ACC AAC TGC TTA GC 3'	5' GGC ATG GAC TGT GGT CAT G 3'
CYCLO	5' GCA GAC AAG GTC CCA AAG 3'	5' GAA GTC ACC ACC CTG ACA C 3'

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